



3 1761 11973444 0



Digitized by the Internet Archive
in 2023 with funding from
University of Toronto

<https://archive.org/details/31761119734440>

Royal Commission on
New Reproductive Technologies



Commission royale sur les
nouvelles techniques de reproduction

CAI
21
-1989
R115

BACKGROUND AND CURRENT PRACTICE OF FETAL TISSUE AND EMBRYO RESEARCH IN CANADA

Research Studies of the
Royal Commission on
New Reproductive Technologies



Contents

Background and Current Practice of Fetal Tissue and Embryo Research in Canada

Volume 15 of the Research Studies

Royal Commission on New Reproductive Technologies

© Minister of Supply and Services Canada, 1993
Printed and bound in Canada

This volume is available in both official languages. Each volume is individually priced, but is also available as part of a complete set containing all 15 volumes.

Available in Canada through your local bookseller
or by mail from
Canada Communications Group — Publishing
Ottawa, Canada K1A 0S9

CANADIAN CATALOGUING IN PUBLICATION DATA

Main entry under title:

Background and current practice of fetal tissue and embryo research in Canada

(Research studies ; no. 15)

Issued also in French under title: Contexte et pratique actuelle de la recherche sur l'embryon et les tissus foetaux au Canada.

Includes bibliographical references.

ISBN 0-662-21389-0

Cat. no. Z1-1989/3-41-28E

1. Embryology, Human — Research — Canada. 2. Fetal tissues — Research — Canada. I. Canada. Royal Commission on New Reproductive Technologies. II. Series: Research studies (Canada. Royal Commission on New Reproductive Technologies) ; 15.

RG133.5B32 1993

612.6'4'0072071

C94-980066-X

The Royal Commission on New Reproductive Technologies and the publishers wish to acknowledge with gratitude the following:

- Canada Communications Group, Printing Services
- Canada Communications Group, Graphics

Consistent with the Commission's commitment to full equality between men and women, care has been taken throughout this volume to use gender-neutral language wherever possible.



AVM 0849

Contents

Preface from the Chairperson
Introduction

ix
xiii

1 The Use of Human Embryos and Fetal Tissues: A Research Architecture

Michelle A. Mullen

Executive Summary	1
Introduction	2
Biological Characteristics of the Human Embryo	3
Biological Characteristics of the Fetus and Fetal Tissues	6
Sources of the Human Embryo	8
Sources of Human Fetal Tissue	9
Medical and Technical Considerations and Indications:	
The Embryo	10
Medical and Technical Applications: Fetal Tissue	15
Alternatives and Limitations: Embryo Research	24
Alternatives and Limitations: Fetal Tissue Research	26
A Schematic Overview: Embryo and Fetal Tissue Research	27
Glossary of Terms	28
Notes	29
Bibliography	35

2 Legal Issues in Embryo and Fetal Tissue Research and Therapy

Bernard M. Dickens

Executive Summary	43
Introduction	44
Treatment of Particular Pre-Embryos, Embryos, and Fetuses	46
Treatment to Benefit Pre-Embryos, Embryos, and Fetuses	49
Treatment to Benefit Patients Through the Use of Embryonic or Fetal Tissues	52
Development of Commercial Interests Through the Use of Embryonic or Fetal Tissues	54
Abbreviations	56
Notes	56
Bibliography	56

3

Human Fetal Tissue Research: Origins, State of the Art, Future Applications, and Implications

Alan Fine

Executive Summary	57
Introduction	58
Relevant Properties of Fetal Tissue	58
Research and Therapeutic Uses of Human Fetal Tissue:	
Review and State of the Art	60
Future Applications of Human Fetal Tissue	74
Implications	80
Acknowledgments	96
References	96

4

Report on a Survey of Use and Handling of Human Reproductive Tissues in Canadian Health Care Facilities

SPR Associates Inc.

Executive Summary	121
Introduction	123
The Survey	124
Uses and Distribution of Human Reproductive Tissues in Canadian Health Care Facilities with Obstetric and Gynaecological Services	127
Uses and Distribution of Human Reproductive Tissues Among Canadian Abortion Clinics	134
Conclusions	134
Appendix 1. Survey Questionnaires	135
Acknowledgments	151
Notes	152

Tables

1. Health Care Facility Survey Responses by Province/Territory	125
2. Health Care Facility Survey Responses by Size of Community	126
3. Uses and Distribution of Ova Among All Health Care Facilities Surveyed	128
4. Uses and Distribution of Embryos Among All Health Care Facilities Surveyed	129

5. Uses and Distribution of Ovarian Tissue Among All Health Care Facilities Surveyed	130
6. Uses and Distribution of Abortuses and Fetal Tissue Among All Health Care Facilities Surveyed	130
7. Uses and Distribution of Placentas Among All Health Care Facilities Surveyed	131
8. Distribution Patterns for Human Reproductive Tissues Among All Health Care Facilities Distributing Human Reproductive Tissues	133

**Report on a Follow-Up Survey of Use and Handling of Human Reproductive Tissues
(Survey of Medical Laboratories and Medical Waste Disposal Firms)**

SPR Associates Inc.

Executive Summary	153
Introduction	155
The Survey	156
Medical Laboratories: Uses and Distribution of Human Reproductive Tissues	158
Medical Waste Firms and Human Reproductive Tissues	161
Conclusions	163
Appendix 1. Survey Questionnaires	164
Acknowledgments	175
Notes	175

Tables

1. Follow-Up Survey	156
2. Response Rates	157
3a. Uses of Human Reproductive Tissues by 48 Medical Laboratories	158
3b. Reasons for Further Distribution of Human Reproductive Tissues	159
4. Research Projects Reported by Responding Medical Laboratories	160
5. Reported Uses of Human Reproductive Tissues by Health Care Facilities Selling Placentas to One Medical Waste Firm	162

◆ 6 Embryo Transfer and Related Technologies in Domestic Animals: Their History, Current Status, and Future Direction, with Special Reference to Implications for Human Medicine

K.J. Betteridge and D. Rieger

Executive Summary	177
Introduction	178
Comparative Aspects of Reproductive Physiology	187
Current Procedures in Embryo Transfer	192
Producing Embryos by <i>In Vitro</i> Procedures	209
Specialized and Experimental Techniques of Embryo Manipulation	213
Future Directions	223
“Technology Transfer” Between the Medical and Veterinary Users of Applied Embryology	226
Conclusions	228
Acknowledgments	229
Notes	229
References	230

Tables

1. Embryo Transfer Activity Reported in Cattle in Europe for the Year 1989	186
2. Pregnancy Rates Obtained with Embryos Classified Morphologically into Three or Four Grades of Descending Quality in Five Separate Studies	198
3. The Procedures and Components of Embryo Transfer and the Associated Factors That Can Affect Embryos Between Collection and Transfer	202
4. A Comparison of the <i>In Vivo</i> Environment of Preimplantation Embryos with <i>In Vitro</i> Culture Conditions	203

Figures

1. Attitudes Evoked by Mammalian Embryos	181
2. The Alteration of Generations	182
3. Patterns of Growth in Embryo Transfer in Cattle in North America Since Commercial Activity Began in the Early 1970s	185
4. Alternative Approaches to Producing More and Cheaper Embryos or Progeny of Desired Genotypes	214
5. The Principles of Nuclear Transplantation for the “Cloning” of Embryos	220

6. Projected Worldwide Growth of Scientific and Technologic Work with Preimplantation Embryos	224
---	-----

Human Embryo Research: Past, Present, and Future

Anne McLaren

Executive Summary	249
Introduction	250
Postimplantation Embryo Research	253
Preimplantation Embryo Research	261
Conclusion	270
References	270

Figure

1. Chronology of Human Prenatal Development	251
---	-----

Preface from the Chairperson



As Canadians living in the last decade of the twentieth century, we face unprecedented choices about procreation. Our responses to those choices — as individuals and as a society — say much about what we value and what our priorities are. Some technologies, such as those for assisted reproduction, are unlikely to become a common means of having a family — although the number of children born as a result of these techniques is greater than the number of infants placed for adoption in Canada. Others, such as ultrasound during pregnancy, are already generally accepted, and half of all pregnant women aged 35 and over undergo prenatal diagnostic procedures. Still other technologies, such as fetal tissue research, have little to do with reproduction as such, but may be of benefit to people suffering from diseases such as Parkinson's; they raise important ethical issues in the use and handling of reproductive tissues.

It is clear that opportunities for technological intervention raise issues that affect all of society; in addition, access to the technologies depends on the existence of public structures and policies to provide them. The values and priorities of society, as expressed through its institutions, laws, and funding arrangements, will affect individual options and choices.

As Canadians became more aware of these technologies throughout the 1980s, there was a growing awareness that there was an unacceptably large gap between the rapid pace of technological change and the policy development needed to guide decisions about whether and how to use such powerful technologies. There was also a realization of how little reliable information was available to make the needed policy decisions. In addition, many of the attitudes and assumptions underlying the way in which technologies were being developed and made available did not reflect the profound changes that have been transforming Canada in recent decades. Individual cases were being dealt with in isolation, and often in the absence of informed social consensus. At the same time, Canadians were looking

more critically at the role of science and technology in their lives in general, becoming more aware of their limited capacity to solve society's problems.

These concerns came together in the creation of the Royal Commission on New Reproductive Technologies. The Commission was established by the federal government in October 1989, with a wide-ranging and complex mandate. It is important to understand that the Commission was asked to consider the technologies' impact not only on society, but also on specific groups in society, particularly women and children. It was asked to consider not only the technologies' scientific and medical aspects, but also their ethical, legal, social, economic, and health implications. Its mandate was extensive, as it was directed to examine not only current developments in the area of new reproductive technologies, but also potential ones; not only techniques related to assisted conception, but also those of prenatal diagnosis; not only the condition of infertility, but also its causes and prevention; not only applications of technology, but also research, particularly embryo and fetal tissue research.

The appointment of a Royal Commission provided an opportunity to collect much-needed information, to foster public awareness and public debate, and to provide a principled framework for Canadian public policy on the use or restriction of these technologies.

The Commission set three broad goals for its work: to provide direction for public policy by making sound, practical, and principled recommendations; to leave a legacy of increased knowledge to benefit Canadian and international experience with new reproductive technologies; and to enhance public awareness and understanding of the issues surrounding new reproductive technologies to facilitate public participation in determining the future of the technologies and their place in Canadian society.

To fulfil these goals, the Commission held extensive public consultations, including private sessions for people with personal experiences of the technologies that they did not want to discuss in a public forum, and it developed an interdisciplinary research program to ensure that its recommendations would be informed by rigorous and wide-ranging research. In fact, the Commission published some of that research in advance of the Final Report to assist those working in the field of reproductive health and new reproductive technologies and to help inform the public.

The results of the research program are presented in these volumes. In all, the Commission developed and gathered an enormous body of information and analysis on which to base its recommendations, much of it available in Canada for the first time. This solid base of research findings helped to clarify the issues and produce practical and useful recommendations based on reliable data about the reality of the situation, not on speculation.

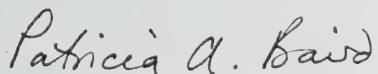
The Commission sought the involvement of the most qualified researchers to help develop its research projects. In total, more than 300

scholars and academics representing more than 70 disciplines — including the social sciences, humanities, medicine, genetics, life sciences, law, ethics, philosophy, and theology — at some 21 Canadian universities and 13 hospitals, clinics, and other institutions were involved in the research program.

The Commission was committed to a research process with high standards and a protocol that included internal and external peer review for content and methodology, first at the design stage and later at the report stage. Authors were asked to respond to these reviews, and the process resulted in the achievement of a high standard of work. The protocol was completed before the publication of the studies in this series of research volumes. Researchers using human subjects were required to comply with appropriate ethical review standards.

These volumes of research studies reflect the Commission's wide mandate. We believe the findings and analysis contained in these volumes will be useful for many people, both in this country and elsewhere.

Along with the other Commissioners, I would like to take this opportunity to extend my appreciation and thanks to the researchers and external reviewers who have given tremendous amounts of time and thought to the Commission. I would also like to acknowledge the entire Commission staff for their hard work, dedication, and commitment over the life of the Commission. Finally, I would like to thank the more than 40 000 Canadians who were involved in the many facets of the Commission's work. Their contribution has been invaluable.

A handwritten signature in black ink that reads "Patricia A. Baird". The signature is fluid and cursive, with "Patricia" on the first line and "A. Baird" on the second line.

Patricia Baird, M.D., C.M., FRCPC, F.C.C.M.G.

Introduction



Embryo research and fetal tissue research have not been as great a focus of public attention and awareness or of data gathering as infertility treatments or prenatal diagnosis, and there is a much lower level of awareness of and information about these activities in Canada. This relative lack of awareness of the issues raised by these areas of research, and of the activities carried out in them, is replicated in the microcosm of hospitals and other medical facilities, with most institution decision makers unaware of or only vaguely informed about what their own institutions are doing in these areas.

This is not to say that embryo research and fetal tissue research have been occurring in a vacuum. Through its guidelines on experimentation involving human subjects, and its more general role in the research community, the Medical Research Council of Canada has been instrumental in influencing the current situation in Canada for both embryo and fetal tissue research. Ethics review boards across the country have dealt on a case-by-case basis with the issues raised by research proposals; an example is the very thorough review of a fetal tissue research proposal in Halifax, first by the hospital involved, then by the affiliated university. In addition, there have been various expert bodies, in Canada and internationally, that have considered the issues raised by such research. The Commission has learned from all these activities, as outlined in some of the papers in this volume, using this knowledge to help provide background for its deliberations.

The two kinds of research — research using embryos and research using fetal tissues — are very different in nature. Embryo research focusses on questions relating to conception and reproduction, and thus is linked directly to the field of human reproduction. Some knowledge about human reproduction critical to treating infertility can be gained only through research using human zygotes. For example, what is the best

culture medium in which to keep eggs so they will fertilize and develop normally? Only if you expose eggs that have been kept in different culture media to sperm and observe whether they fertilize and divide normally can you discover this. This kind of knowledge is needed to ensure the safety and quality of *in vitro* fertilization and related medical treatments. At the same time, however, embryo research deals with an entity that may have the potential for life if implanted, so it is also essential to ensure that zygotes are treated with respect because of their connections to the human community.

Fetal tissue research is based on the unique biological properties of tissue derived from a dead fetus, and one avenue of research that has been identified is the possibility that fetal tissue can be used in transplantation to help people who have certain kinds of diseases or disorders. What is learned from this research will be applicable largely outside the field of reproduction. However, because the only practical source of fetal tissue at present is elective abortions, an important aspect to be addressed is whether allowing this research would encourage more abortions.

Some embryo research is needed if safe and effective assisted conception services are to be provided, but it should take into account the ethical issues involved. The future of fetal tissue research is difficult to predict, because we do not yet know how effective fetal tissue transplantation will be as therapy. If it demonstrates promising results in treating disease, particularly neurological disorders, there will be a call for increased use of such tissue. This underscores the importance of introducing mechanisms to ensure such tissue is obtained and used only in an ethical and accountable way.

The Studies

Michelle Mullen's examination of human embryo and fetal tissue research provides a concise overview of two areas of new reproductive technologies that are characterized both by differences and by some similarities. She reviews the special biological properties of the human embryo and the fetus and their sources for use in research. She gives an overview of current medical and basic scientific research applications, as well as limitations of their use. She outlines differences between the two areas of research, but also notes that social, ethical, and legal issues arise in both.

Bernard Dickens takes up this theme with a comprehensive survey of the legal issues associated with human embryo and fetal tissue research. In his analysis, he differentiates between research for the benefit of a particular pre-embryo, embryo, or fetus; for the benefit of pre-embryos, embryos, or fetuses in general; for the benefit of patients through treatment using such tissues; and for the development of commercial interests through the use of such tissues.

In some cases, the legal issues will require novel application of existing legal principles to the new situations created by the process of obtaining and manipulating embryos and fetal tissue. He notes that, in other cases, there will be an increasing need for new approaches to the very complex and potentially controversial issues generated, particularly by commercial interests in embryo or fetal tissue research.

Alan Fine gives an overview of the origins, current practice, and future applications of human fetal tissue research. Laboratory studies suggest that a wide range of disorders may be treatable in the future by transplantation of various types of human fetal tissue. Fetal tissue is uniquely valuable for such transplantation, but, at the same time, there is a need to ensure any use of such tissue does not encourage abortions. If there were to be significant increases in demand for fetal tissue in the future, it would be important to have in place clear mechanisms for ethical and accountable means of obtaining, distributing, and using this tissue. Dr. Fine concludes that these issues can and should be addressed through appropriate regulation and that they need not be an impediment to legitimate research and therapeutic use of fetal tissue.

SPR Associates carried out a survey for the Commission of Canadian health care facilities where human embryos and fetal tissue might be obtained, to find out about the use and handling of human embryo and fetal tissue. More than 650 hospitals offering obstetric and gynaecological services and abortion clinics were surveyed, with a response rate of more than 90 percent. SPR Associates describe a widespread lack of awareness of issues surrounding reproductive tissues and a general lack of formal protocols and written guidelines relating to the handling of such tissues. Among the facilities that did report having written protocols, there were few consent forms noted in the documentation provided. Few facilities retained the tissues for in-house research, but many of the administrators completing the survey did not know the actual end use made of tissue sent to recipients, and internal tracking of the tissues was less than satisfactory.

Given these findings, SPR Associates conducted a follow-up study of recipients — medical laboratories and medical waste disposal firms — as a way of finding out what uses were being made of embryo and fetal tissues obtained from Canadian health care facilities. As SPR Associates report, almost all tissue received by medical laboratories was used for pathology examination, while that received by medical waste companies was disposed of. One exception was placentas, which were collected by one company and then sold to a French institute for the production of pharmaceutical products.

While the follow-up survey confirmed that the major use of these tissues was related to pathology examination, it was able to identify 19 research projects that had not been identified by the health care facilities in the previous survey — despite the fact that often the recipient laboratory was affiliated with the major hospital that had responded to the previous survey. This is an indication that information flow is not as it should be,

and it is clear that respondents answering for health facilities are not always aware of the exact use of these tissues, within either their own institution or an affiliated laboratory.

Some public concern about new reproductive technologies in general, but about embryo research in particular, relates to the potential transfer of technology from animal husbandry to humans. There is concern that goals, values, and attitudes appropriate to animal husbandry but not to human medicine could be transferred as well and have an adverse influence on how assisted reproductive technologies develop and are used in people. As Keith Betteridge and Donald Rieger note, some technical aspects are similar for humans and domestic animals, and the increase in our understanding of mammalian reproduction resulting from technologies used in domestic animals has been of benefit to humans. They clarify, however, the very different objectives of assisted reproduction in the human and animal fields. In humans, the purpose of reproductive manipulation is to benefit the individual, while in domestic animals technology is used to improve production, to the benefit not of the animal, but of the farmer and, ultimately, of the consumer. They further point out that differences in biology also mean that some technologies cannot be directly transferred. For instance, the "mass production" of domestic animals that can be achieved using new technologies is partly possible because the characteristics the embryos will have are known in specially bred animals, whereas this is not possible in humans. Another difference is the long period between fertilization and implantation, whereas this period is very much shorter in humans.

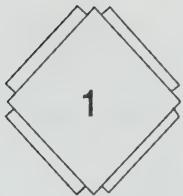
The shorter preimplantation period in humans also has implications for embryo research. Anne McLaren's overview of human embryo research benefits from her familiarity with the situation in the United Kingdom, where there has been extensive public debate about this kind of research. She separates the research into two categories, preimplantation and postimplantation embryo research, in recognition of the fact that there are significant developmental changes after implantation. Preimplantation research, in particular, is aimed at developing a better understanding of infertility, in the hope of being able to alleviate it, at understanding genetic and chromosomal defects, which may account for reproductive failure at this early stage, and at diagnosing severe genetic disorders in the zygotes of high-risk couples prior to implantation.

In addition to providing a survey of key research trends in both pre- and postimplantation embryo research, Dr. McLaren examines the social, legal, and ethical issues the research raises and the regulatory schemes that have been put in place in the United Kingdom to deal with these issues.

Conclusion

The studies in this volume lead to the conclusion that most of the small amount of research that is being done in Canada using zygotes or fetal tissue is being done in a way that is relatively invisible to health care facility administrations and without written guidelines or reporting. The result is that there is relatively little public information or accountability in an area that generates profound ethical, social, and legal issues and concerns.

These findings suggest that Canadian society as a whole receives less information than it probably should on a regular basis about both human embryo and fetal tissue research. They underscore the pressing need for public regulation and accountability in the areas of embryo and fetal tissue research. As Alan Fine concludes, "These issues should be resolved deliberately, not by default," and there is a need for clear and accountable public policy in this area.



The Use of Human Embryos and Fetal Tissues: A Research Architecture

Michelle A. Mullen



Executive Summary

This paper reviews the special biological properties of the human embryo and fetus, their sources for research, and current medical and basic scientific research applications. Clinical and scientific applications are considered according to their relevance to a clinical model: thus clinical research, pre-clinical research, and basic research represent the primary, secondary, and tertiary levels of this research taxonomy. Clearly, there are other models for organizing information — for example, in relation to disease type. The research architecture used in this paper is chosen to underline clinical and social relevance for public policy analysis. Limitations in the use of embryos and fetal tissues have been highlighted.

Certain unique biological properties, especially of fetal tissues, have fostered intense interest at both the clinical and the basic research levels. The growing list of current and potential experimental applications for human embryos and fetal tissues suggests the possibility not only of supply and demand conflicts, but also of a range of social, legal, and ethical issues to be addressed.

Embryo research may raise questions of who is being treated — the couple who suffers infertility or carries genes for serious hereditary diseases, or the embryo itself. The distinction between being a patient and being an experimental subject may also be unclear. Further, competition in the use of scarce embryos for clinical and basic research may arise. Issues of informed consent for couples pursuing pregnancies that incorporate clinical embryo research are critical, and the role of such couples in providing proxy consent on behalf of their potential offspring for embryonic experimentation may pose many new and difficult ethical questions.

Current fetal tissue research indicates a growing list of possible therapeutic applications, including Parkinson's and Alzheimer's diseases, diabetes, and AIDS. Fetal tissues may become increasingly scarce as the scope of application widens and as earlier abortion techniques are developed. Currently, there is no agreement about whether the use of electively aborted fetal tissues can be separated from the act of abortion, morally or procedurally. This represents an area for further investigation and discussion.

Finally, an important limitation to this paper is that it reviews only those uses of embryos and fetal tissue reported in the academic and medical literature. Questions of whether and how embryonic and fetal tissues are used in the cosmetic, pharmaceutical, and other industries have not been examined. Social, ethical, legal, and regulatory issues have not been fully addressed. These are important domains of original research and discourse if effective and relevant public policies are to be developed.

Introduction

As areas of academic inquiry, the use of human embryos for research purposes (ER) and the use of human fetal tissue for therapeutic research (FTT) share several features. Both are interdisciplinary subjects, requiring a consideration of philosophical premises, medical and technical parameters, legal context, and ethical concerns. This relatedness is most significant at the normative or philosophical level of inquiry; debate concerning when human life begins, as well as the rights of the embryo and fetus, are central to the philosophical context.

The specifics of examining the medical and scientific uses of embryos and fetuses lead to quite separate lines of investigation, however. This paper briefly examines embryo research and fetal tissue research in terms of

1. the distinctive biological properties of embryonic and fetal material;
2. sources of these materials;

3. medical and technical applications — in both clinical and basic research terms; and
4. alternatives to these materials in clinical and basic research modalities.

This paper embodies only a brief review of a rich and extensive literature in these areas. Finally, it provides a schematic research architecture intended to highlight the principal lines of scientific and clinical inquiry, as well as key sub-issues to be addressed.

It is useful to clarify some definitions of embryo, fetus, and fetal tissue at the outset. The zygote, cleavage, and blastocyst stages are best regarded as "pre-embryonic," and the term "embryo" technically applies to the structure that is present from the second through the eighth week after fertilization. Currently, most "embryo" research in fact involves the pre-embryo: the properties of totipotentiate development and preimplantation status are most relevant to technologies of assisted reproduction, genetic manipulation, development of cell lines, and "twinning" procedures. This paper details the distinctive features of the pre-embryo and illustrates relevance to the various scientific and clinical procedures that are performed.

It is equally important to note that this paper deals with the use of fetal tissues for research, rather than with fetal research. Fetal tissue research involves the investigation of fetal tissue properties and possible applications in therapy, transplantation, or industry. Clearly such research is not intended to benefit the fetus from which the tissues are derived. By contrast, fetal research is directed at understanding the growth and development of the fetus in both healthy and pathological conditions; this research has resulted in fetal therapies, such as intrauterine fetal surgery and the management of fetal cardiac arrhythmias *in utero*.

Biological Characteristics of the Human Embryo

Certain unique properties of the embryo are of great value in basic and applied research. These characteristics change with the gestational age of the embryo, and it is useful to outline these changes and their significance.

Conception and Zygote Development

The processes that lead from the uniting of human germ cells — ovum and sperm — to the implanted, or true, embryo are continuous and complex. The process can be divided into stages: the new generation begins with the zygote, or newly united egg and sperm. Entry of a single sperm cell into the ready oocyte is itself made possible only by a series of biochemical and physiological alterations in both germ cells: the ovum continues to mature after rupture from the ovarian follicle and is optimally

receptive to fertilization (*in vivo*) some 6 to 12 hours after ovulation, or after artificial retrieval (for fertilization *in vitro*). Similarly, the sperm cell must undergo the changes involved in capacitation (molecular changes undergone by sperm cells after ejaculation that permit the sperm cell to respond to substances accompanying the ovum) and acrosomal reaction (fusion of the acrosomal sites on the sperm cell to allow the formation of "portals" and hence the biochemical release necessary for passage into the ovum).

The response of the egg to penetration is activation, signifying the initiation of embryonic development and incorporating both functional and structural changes in the newly fertilized egg. These include the induction of blockade to polyspermy (permeability loss in the zona pellucida to prevent entry of more than one sperm cell), opening and evacuation of cortical granules, emission of the second polar body, and formation of the two pronuclei, each with its discrete package of genetic information contributed by the parent cells.¹ These subtle biochemical and structural processes are, as yet, only dimly understood, and this "cross-over" time (between dealing with separate germ cells and dealing with the newly fertilized ovum) is an area of great research interest, particularly in terms of understanding fertilization failure (both male and female factors) and errors of fertilization (such as polyspermy).

The Genome

The completion of the union of sperm and egg (zygote formation) and the development of a new nucleus fusing the genetic contribution of the parent germ cells constitute the new hereditary generation, with its new genome. The zygote is remarkable in its theoretical potential to give rise to a distinct and unique member of the human community. This potential is both theoretical and statistical, because only about one in three zygotes created *in vivo* will accomplish this, and the potential is conditional on successful uterine implantation.²

The information contained in this new genetic entity is replicated in each somatic cell in the developing embryo and in each cell of the human being that may result. The identical replication of the genome in each cell is essential to the study of genetic markers, whether at the stage of preimplantation embryo, during prenatal diagnosis (chorionic villus sampling and amniocentesis), or after birth.

Cleavage and the Blastomere

The advent of the new genome is followed by the process of cleavage. During this time the initial single cell and its nucleus divide successively: one cell becomes two, then four, then eight, and so on. Each of these successive equal divisions occurs with little or no intervening growth in the overall mass of the developing entity. Thus each successive product cell

(blastomere) becomes increasingly smaller as the size of the total aggregate remains nearly constant throughout the early stages of division.³

Totipotentiality

For a given individual, nearly all somatic cells contain the identical genome, or hereditary information, but the cells of the early pre-embryo are equipped with a unique property — totipotentiality. Stated simply, each cell in the early stages of division has the theoretical potential to develop into a full adult. This property has implications for twinning and fusion and for the biopsy of the early embryo for diagnosis. This property endures through the eight-cell stage, when all the blastomeres are equally potentiate for further development. By the 32-cell stage this totipotentiate quality is lost, and as cell numbers increase, differentiation into particular cell types occurs.

Twinning and Fusion

Animal experiments using mouse embryos up to the eight-cell stage have demonstrated that each blastomere has the potential to develop into a complete adult, if separated from the other cells. This phenomenon may occur naturally, as in the case of human identical (monozygotic) twins. Thus the very early human pre-embryo has the potential to become none, one, or more than one distinct human being.⁴ Similarly, experiments in the mouse have demonstrated that if two eight-cell embryonic aggregates of different parentage are fused, then a single adult may result. Genetic contributions from four parents can be recognized in the resultant individual in fusion experiments.⁵ Thus at the eight-cell stage, the developmental singleness of one individual person has not yet been established. It may be inaccurate to speak of the early embryo, which is neither singular nor plural.

Implantation and Differentiation

Within two divisions of the eight-cell stage, at 32 cells, totipotentiality is lost. The cells have become more adherent and are densely packed. With increasing cell numbers, multi-cellular forms can be identified (differentiation) — certain cells forming an outer layer surrounding a less differentiated inner cell mass. The outer cells are developing into the trophoblast or feeding layer. This material will become extra-embryonic and is engaged in completing the placental interactive layer with the maternal uterus. The inner cell mass may continue to develop into the true embryo. The whole of this continually developing mass is termed the blastocyst; the cavity is termed the blastocoele.⁶ *In vivo*, it is at about this stage that the entity completes its travel, entering the uterus where implantation may occur.

Growth Factors

The very early embryo is not inert with respect to its environment, either *in vivo* or *in vitro*. Studies reveal that the pre-embryo produces and releases growth factors and other chemical messengers from at least the four-cell stage. Identified substances include beta-human chorionic gonadotropin (beta-hcg — the diagnostic pregnancy hormone) and embryo-derived platelet activating factor. The role of various factors is poorly understood; however, certain released factors appear to be necessary for successful implantation in the uterine wall.⁷ Identifying and understanding the functioning of growth and implantation factors derived from the early embryo remain an important area of infertility and implantation research.

Biological Characteristics of the Fetus and Fetal Tissues

This section examines the scientific basis for the desirability of fetal tissue for research and therapeutic purposes, especially the use of fetal tissues for transplantation. The relevant properties of fetal tissue are described in detail.

Differentiation/Dedifferentiation

Fetal tissue cells exhibit a remarkable capacity for growth and differentiation. This is particularly true of early fetal cells, as rudimentary organ functions are developing, and the capacity diminishes with increasing gestational age of the fetus.⁸ This is in distinct contrast to adult human tissues, which exhibit very little capacity for redifferentiation. This property of cells derived from fetal tissues enhances their potential for functional differentiated growth *in vivo* and *in vitro*.

Culture *In Vitro*

The great potential for growth of certain fetal cell types *in vitro* is directly related to the relatively undifferentiated status of those cells. Again, this is in contrast to cells derived from solid adult organs; cells derived from some of these organs may be completely resistant to culture *in vitro* — brain and cardiac cells are examples — while others, such as liver cells, will undergo some replication under appropriate culture conditions. That fetal cells can be cultured suggests research directions in the development and maintenance of specific fetal cell lines in culture, with cryopreservation of these cells as a potential source for continued research and use for therapeutic purposes.⁹

Potential for Growth and Restoration of Function in the Host

Animal models have been used extensively to study the growth and functional capacity of transplanted fetal cells of various types.¹⁰ The results of these studies indicate that there is demonstrable and clinically significant growth and functional recovery by transplanted fetal cells in disease models for Parkinson's disease and Type I (juvenile, or insulin-dependent) diabetes. Such studies provide the rationale for clinical experimental trials using fetal tissue transplantation in human patients when conventional therapies fail.

Resistance to Oxygen Deprivation

The sensitivity of post-natal solid organs to oxygen deprivation is an important limiting factor in transplantation; organ harvest and transport must be carefully timed if the grafting procedure is to have any chance of success. Primitive fetal cells exist under uterine conditions with substantially limited vascularization and oxygenation, and consequently have increased resistance to lack of oxygen at harvest and transplantation.¹¹ Some investigators report that human fetal neural tissue is functional after several hours in saline solution at room temperature. This property renders fetal tissues a particularly attractive source of transplant material.

Ease in Transplantation

Compared with the technical demands of adult solid organ transplantation, the handling of fetal cells for transplantation is remarkably easy. Solid organs demand an intricate surgical approach, particularly in their dissection from vasculature, when harvested for transplantation. Fetal cells can be grossly dissected by cell type, then mechanically manipulated into suspension. This allows transplantation by virtual "injection" into the target host organ and requires no efforts to connect the cells to an existing blood supply.¹²

Immunogenicity

Perhaps the most important obstacle to successful clinical outcome in all transplant procedures is the rejection of foreign transplant material by the recipient. The host immune system recognizes the transplanted material as genetically distinct — as it does with infectious bacteria or viruses — and initiates a destructive response. Transplant outcomes have been dramatically improved by the use of more advanced tissue typing and immuno-suppressive drugs such as cyclosporin. Immuno-suppression is not always successful, however, and serious side-effects are not uncommon; tissue and organ rejection remains a serious clinical challenge. Fetal tissues, by contrast, are characteristically immunologically immature

and may provoke little or no immune response in the host.¹³ This feature of fetal tissue is key to its desirability as a transplant material.

Supply

The scarcity of human organs and tissues for transplantation and treatment represents a grave challenge to health care professionals and patients awaiting transplant. First-trimester abortion is a common procedure in Canada; currently, the uterine contents are disposed of. First-trimester elective abortion represents a potentially vast pool of tissues that might be harvested for therapeutic purposes.¹⁴

Sources of the Human Embryo

In Vitro Creation of Embryos — Assisted Reproductive Technologies

Human pre-embryos for research are generally created *in vitro* in clinical reproductive biology units (both free-standing clinics and hospital- and university-based infertility treatment centres). In these contexts, embryos for research purposes arise in two ways. First, embryos may be designated as “surplus” where ova and sperm have been united in laboratory conditions in the hope of creating embryos suitable for transfer to the uterus of an infertile woman. Ovarian hyperstimulation by drugs such as Clomid®, Pergonal®, Profasi®, and Metrodin® may result in the creation of too many ova for safe transfer (an upper limit of four embryos is considered reasonable to ensure maternal and fetal safety).¹⁵ Depending on the clinical program, surplus embryos may then be designated for cryopreservation (for implantation in a future cycle), donation to another patient, research, or disposal.

A further source of human pre-embryos for research results from the rejection of these embryos for embryo transfer, cryopreservation, or donation because of an obvious defect. Such defects include polyspermy (as demonstrated by the microscopic appearance of more than two pronuclei within 24 hours after insemination) and damage (rupture) of the zona pellucida with cytosolic leakage.¹⁶ Such defects contraindicate embryo transfer and are a potential source of embryos for use in research. Investigation of these pre-embryos may help elucidate the mechanisms of fertilization errors and other disruptions contributing to infertility.

Second, embryos can be created for the specific purpose of research, where donors consent to their gametes/embryos being used in this way. This situation is less common, owing to the relative scarcity of gametes for persons undergoing infertility treatment. However, certain patients who do not wish cryopreservation of spare embryos, or where cryopreservation or

donation is not available, may consent to the use of their excess embryos for research purposes.

Uterine Flushing and Embryo Harvest

Human pre-embryos can be non-surgically "flushed" from the uterus of a newly pregnant woman, less than 14 days after fertilization.¹⁷ These techniques were first developed for the recovery of early embryos in agricultural breeding stock, for subsequent transfer to less valuable stock for gestation. Only a limited number of uterine flushings have been performed in humans. The application of this technique theoretically includes very early embryonic diagnosis of genetic defects in high-risk couples — for example, carriers of the cystic fibrosis gene. It should be noted, however, that guidelines developed by the Society of Obstetricians and Gynaecologists of Canada recommend against the use of uterine flushing for retrieval of ova and embryos for the purpose of donation, because of the risks to a woman undergoing this procedure.¹⁸

Selection for Cryopreservation

Excess embryos created *in vitro* by assisted reproductive technologies may be selected for cryopreservation, based on gross morphological characteristics including cell number, equal blastomere size, and lack of fragmentation. A confirmation of normal fertilization by visualization of two pronuclei on the first day after insemination is necessary to exclude polyploidy. Embryos failing to meet these criteria are rejected for freezing and are a potential source of research embryos.¹⁹

Sources of Human Fetal Tissue

Therapeutic abortion, spontaneous abortion (miscarriage), and ectopic pregnancy are all potential sources of fetal tissue. Whatever the source, fetal tissue must be viable, free from major genetic defects, and uncontaminated by infectious agents — bacterial, viral, or fungal — to be suitable for transplantation and most basic research studies. These requisite features must be considered when evaluating the potential sources.

Therapeutic Abortion

The usual source of human fetal tissue for research and therapeutic application is the elective first-trimester abortion. In the literature, details are scant with respect to the administrative aspects of collecting this material; many hospital consent forms for the procedure contain a global clause, in rather general terms, permitting the use and/or disposal of the abortus material. It is known that up to 1.6 million abortions are performed annually in the United States and up to 85 000 in Canada; about 90 percent of these take place within the first trimester.²⁰ Vacuum

aspiration is considered the method of choice for first-trimester abortion at this time. During the second trimester, dilatation and evacuation are usually employed; this method results in whole fetus recovery, while the vacuum aspiration approach causes significant fragmentation of the contents of pregnancy. Very late abortions can be performed by replacing the amniotic fluid with concentrated saline and stimulating uterine contractions with pitocin or prostaglandins.²¹

Spontaneous Abortion

Another potential source of fetal material for research is spontaneous abortions (miscarriage). Difficulties in procuring this material include loss of control over timing of harvest, fetal death and tissue necrosis, and concern about the genetic normalcy of spontaneous abortions. Additionally, infections such as syphilis, rubella, and mycoplasma may cause spontaneous abortion.²² For these reasons, fetal tissue from spontaneous abortion is not used for research or clinical applications.

Ectopic Pregnancy

Fetal tissue derived from ectopic pregnancies has been suggested as a practical and ethical source of this material. Between 40 percent and 64 percent of ectopic pregnancies abort spontaneously in the first trimester; this abortus material is rarely recognizable or viable in culture.²³ The balance of ectopic pregnancies become clinically apparent before the ninth week of gestation, and intervention is necessary to save the mother from this life-threatening condition. This intervention may involve surgical removal of the gestation; more recently, however, non-surgical local injection of methotrexate (lethal to the fetus) has been used, so as to preserve the fallopian tube for future reconstruction.²⁴ This injection procedure renders the fetal tissue unsuitable for research.

Medical and Technical Considerations and Indications: The Embryo

This section constitutes the major portion of this paper. Differences between the issues pertinent to the use of human embryos for research purposes and the use of human fetal tissue for therapeutic research are more salient when clinical and research applications are considered. Embryo research, in general, is directed at medical and scientific inquiry relevant to human reproduction, so that, at least potentially, it may serve to benefit the larger population of human embryos, if not the specific embryo as research subject. By contrast, fetal tissue for therapeutic research is generally, but not exclusively, directed toward therapy for adults.

Primary Applications — Assisted Reproductive Technologies

The development of new assisted reproductive technologies has relied extensively on human pre-embryo research. While animal models were used for many years to investigate the mechanisms of mammalian fertilization *in vitro*, the advent of *in vitro* fertilization-embryo transfer as a clinical treatment for infertility has fostered a peculiar environment where infertility research and treatment take place simultaneously. Examples of this include the application of modified techniques such as gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT), and pronuclear oocyte salpingo transfer (PROST).²⁵

Quality Control

Quality control is an essential feature of infertility laboratories processing human gametes and pre-embryos. Rigorous laboratory methods demand daily monitoring of *in vitro* culture conditions, including temperature, pH (relative acid/base index of the culture environment), humidity, sterility, and quality of culture media. Where more than one incubator is available, comparing parameters from one machine to the other provides an internal quality control. (It is noteworthy that availability of more than one incubating chamber is an important precaution in human embryo culture, so that an immediate back-up is available in the event of a system failure.²⁶) In this sense, all human gametes and pre-embryos in a given clinical culture setting exist as internal controls for one another. In a given laboratory, fertilization failure with one set of gametes may be more confidently ascribed to the inherent properties of those gametes when germ cells from other patients fertilize and develop successfully. When all patients' gametes fail to fertilize or undergo cleavage, this points more strongly, though not exclusively, to failure of the culture conditions.

Culture Media

Many programs employ simultaneous assay of animal culture as a measure of quality control, particularly in the preparation of culture media, with mouse embryo the most common model. Subtle but critical problems with media may be detected this way: in one example, quality control tests demonstrated that a commercially available culture medium allowed normal development of two-cell mouse embryos, while one-cell mouse embryos (pronuclear oocytes) failed to undergo cleavage.²⁷ Such results point to the highly sensitive requirements for successful culture of mammalian embryos and the need for continuing research into improved and reliable culture techniques.

Cryopreservation

The cryopreservation of human embryos represents an additional area of ongoing primary research in infertility treatment. The creation of supernumerary embryos in a given *in vitro* fertilization treatment cycle results from current ovarian stimulation approaches. Morbidity and mortality associated with multiple pregnancy have led to policies

recommending the transfer of only three embryos on a given cycle, with a maximum of four under exceptional conditions. Successful freezing, thawing, and transfer of human embryos may potentially serve to improve assisted reproductive technology results for a given ovarian stimulation treatment.

Some findings suggest that pregnancy results are improved when embryos are transferred at the appropriate time in a "natural" or non-exogenous ovarian stimulation cycle. Thus the patient may benefit from increased chances of pregnancy with fewer exposures to exogenous ovarian stimulation and invasive oocyte retrieval, fewer associated medical risks, and lower financial cost.²⁸ The results of embryo cryopreservation programs around the world vary, however. Australian results indicate a significant improvement in continuing pregnancies when frozen and thawed embryos were transferred during the natural cycle when compared with embryo replacement during a stimulation cycle.²⁹ A recent report from the Chaim Sheba Medical Centre IVF Program in Israel found that pregnancy potential is diminished using frozen embryos.³⁰ Research activities in this area include the comparison of different cryoprotectants (dimethyl sulphoxide [DMSO] and 1,2-propanediol) and staged temperature-lowering protocols with rapid and ultra-rapid freezing techniques.³¹ Again, investigations into improved methods of embryo freezing in various treatment programs can be seen as a simultaneous embryo research and infertility treatment endeavour.

Male Factor Infertility

Finally, novel clinical approaches aimed at improving fertilization and pregnancy rates where poor sperm quality is a problem illustrate another area of simultaneous treatment and research. "Male factors" as the sole cause of infertility account for at least 25 percent of infertility experienced by couples and are implicated in at least 40 percent of infertile couples as a co-factor in infertility. *In vitro* fertilization has been used for a number of years to treat male factor infertility, as fertilization with certain male factors is improved *in vitro*, especially where poor sperm motility is present. Clinical research in this area centres on the development of techniques to facilitate fertilization. One approach has been the development of differential chemical gradients to select the healthiest sperm for insemination *in vitro*, while other methods employ disruption (by drilling or fracture) of the ovum zona pellucida to ease entry of sperm. Micro-injection of a single sperm cell into the cytoplasm of the ovum has been attempted.³² It is noteworthy that the subtle effects of these manipulations on the health of the potential child and adult cannot yet be known, although some apparently successful pregnancies and deliveries have resulted following the use of each of these methods.

Secondary Research — Embryo Growth

There is great interest in elucidating the subtle mechanisms of embryo growth, differentiation, implantation, and immunology for the purpose of elaborating existing theoretical knowledge and discovering improved approaches to infertility and healthy reproduction.

Embryo-Released Factors

Very little is known about the factors released by the early embryo and the nature of their function in development and interaction with the maternal environment (*in utero*). Human pre-embryos manufacture and release both beta-hcg and platelet activating factor in culture, although the reasons are still not understood. It is known that the embryo binds glycoproteins from its environment as it travels along the fallopian tube; again, the roles of these phenomena are matters of speculation. Insulin appears to have a growth factor effect on pre-embryos in certain culture conditions. The *in vivo* effects of circulating drugs from exogenous ovarian stimulation on the newly developing pre-embryo are not known.³³

Identification and functional analysis of pre-embryonic growth factors and chemical messengers may provide clues to understanding "unexplained" infertility and may help to define subtle parameters necessary for normal growth and interaction with both culture and uterine environments. Preliminary research has begun to reveal the metabolic behaviour of human pre-embryos through the development of assays to show how much and what type of nutrient the embryo takes up from culture media.³⁴

The mechanisms of differentiation from totipotent pre-embryonic cells (fewer than 32 cells) into placental interactive tissue and into undifferentiated true embryonic cell mass remain largely elusive, as do the triggers for the differentiation of the inner cell mass into specialized tissues and then organs during gestation. Elucidating these mechanisms is of great interest to basic biologists and those scientists involved in normal and abnormal human reproduction. Currently, only certain gross morphological characteristics of the pre-embryo have been correlated with observed healthy development of a human life.³⁵

Implantation

A final area of secondary human pre-embryo research centres on the mechanisms and necessary conditions for successful implantation of the embryo in the uterine wall. These investigations may prove instrumental in understanding failed implantations and the relatively high early embryonic waste seen in humans. Further, understanding implantation may help explain ectopic pregnancies where no obvious tubal or structural defect can be demonstrated.³⁶ Ectopic pregnancy remains an important clinical problem in gynaecology, in terms of both acute patient management and the future fertility of a woman who experiences an ectopic pregnancy.

Tertiary Research — Embryo Biology

One of the most dramatic advances in the area of basic embryo biology has been the very recent development of techniques for preimplantation diagnosis of genetic composition and disease in the human pre-embryo.

Preimplantation Genetic Diagnosis

Preimplantation genetic diagnosis is an experimental diagnostic technique designed for application to embryos that may be replaced in the uterus. Very early embryos contain cells of equal potential development, and it is possible to remove at least one cell (blastomere) from a four-cell or eight-cell pre-embryo, without compromising the normal development of a pregnancy, if the "biopsied" embryo is transferred to a receptive uterus.

The method requires removal of the zona pellucida, washing the embryo in a calcium-free medium to loosen cellular adherence, then gently lifting away one cell. In a mouse model for this research, biochemical assay was performed to determine the presence of the gene-product enzyme HPRT. Results of the assay were available in 24 hours, and the biopsied embryo was transferred successfully.

HPRT deficiency is a sex-linked genetic defect in humans causing a severe neurological disorder (Lesch-Nyhan disease) in affected males.³⁷ It is also possible to freeze the biopsied embryo until such time as laboratory tests of the collected cell are complete, should test results take more than 24 to 48 hours. The embryo can then be thawed and transferred. Thus application of this procedure may well necessitate the incorporation of embryo cryopreservation techniques. The method could be applied to embryos created *in vitro* or harvested by uterine lavage for the purpose of genetic diagnosis.³⁸

Genetic Diagnosis

The development of preimplantation diagnosis for genetic defects will undoubtedly accelerate the debate on social and ethical issues relevant to genetic testing. Some may feel that preimplantation testing and selection of only healthy pre-embryos for uterine transfer would be less traumatic — physically, psychologically, and socially — than current methods of prenatal diagnosis and subsequent abortion of defective fetuses.³⁹

In 1989, for example, the gene for cystic fibrosis was identified.⁴⁰ Cystic fibrosis is the most common genetic disease affecting Caucasians; the potential for preimplantation diagnosis may become a very attractive option for couples where both are carriers but wish to have children. The same procedure may be possible in the future for the many inherited diseases for which the gene has yet to be identified. At this time, preimplantation diagnosis requires biopsy and embryo freezing, because the biopsied test cell must be cultured to generate sufficient tissue to identify the appropriate deoxyribonucleic acid (DNA).⁴¹ Application of these techniques is likely to be severely limited, however, since they require couples to undergo assisted reproduction.

Sex Selection

Preimplantation diagnosis has been used in a limited number of cases to ascertain the sex of the pre-embryo, so as to identify embryos at risk for sex-linked diseases such as muscular dystrophy and haemophilia. A number of sex-linked genetic diseases have been identified; females are generally carriers for these diseases, and affected offspring are generally male.⁴² Identified male embryos from couples at risk would not be implanted; only female embryos would be replaced in the uterus. It is clear, however, that this technique could also be used to select for sex alone in healthy embryos, where there is a strong preference for a child of a specific sex.

Gene Therapy

The ultimate application of preimplantation diagnosis for genetic disease would entail the correction of the defect. Gene therapies unheard of only a few years ago have been developed for specific diseases and are at the stage of clinical trial in a number of models.⁴³ Preimplantation genetic correction has not been attempted, however, and remains a remote possibility at this time. There is little motivation to develop these techniques, because it is possible simply to implant embryos found free of disease, rather than risk transfer of an embryo that has been genetically manipulated. One possible advantage of attempting gene insertion and therapy with the preimplantation embryo is that there are far fewer cells to be manipulated, each of which has great potential for differentiation and proliferation. However, given the choice of implanting only normal embryos, this is a research avenue unlikely to be pursued.⁴⁴

Medical and Technical Applications: Fetal Tissue

The use of human fetal tissues for research, industry, and therapy is not new; however, there has been rapid growth in the potential uses in recent times. Primary applications are those in established use and include vaccine development, viral research, and clinical application. Secondary uses of fetal tissue are clinical treatments where a considerable volume of animal research has led to proposals for or initiation of preliminary clinical trials. Examples are the use of fetal tissues for transplantation in Type I diabetes and Parkinson's disease. Tertiary research includes those possible clinical uses of fetal tissue that are in the earliest stages of investigation.

Primary Applications

Industry

Human fetal tissue in culture has been used for many decades by pharmaceutical and biotechnology companies in the development of vaccines and to test the efficacy and teratogenicity of new pharmaceutical

products.⁴⁵ The human polio vaccine was developed in the 1950s using fetal tissue cells in culture. The ability of fetal cells to divide rapidly and proliferate in culture, their decreased immunogenicity, and their capacity to grow if transplanted into a host are important properties in the selection of fetal tissue for industrial applications. It is noteworthy that the literature in this area is very scant: a search of the International Pharmaceuticals Research Data Base for the past 10 years revealed abstracts related to the effects of drugs on the fetus, but none on the use of fetal tissues in research. It is tempting to speculate that industrial research using fetal tissues is rarely published, possibly owing to the controversial nature of using these tissues (especially if the source is aborted fetuses). By contrast, the academic literature (MEDLINE) on the use of fetal tissues is substantial, perhaps as a result of the onus placed on academic researchers to publish the results of their investigations.

Viral Research

Fetal tissues are used extensively in the investigation of viruses. The rapid proliferation of fetal cells in culture permits rapid replication of a virus for detection, genetic assay, genetic manipulation, and tests of viral facilitators and inhibitors. Such research is performed on human influenza viruses, hepatitis B virus, the measles virus, and human immunodeficiency virus (HIV).⁴⁶ Fetal cells used include those derived from lungs, hepatocytes (early liver cells), and thymus. Fetal tissues are a critical tool in viral research into these clinically important infections.

Treatment of DiGeorge's Syndrome

This syndrome is characterized by a congenital absence of the thymus. The use of human fetal thymus is the only "routine" application of fetal tissue for transplantation; all other transplant procedures using human fetal tissue remain highly experimental. For more than 20 years human fetal thymus transplant has been recognized as the treatment of choice for DiGeorge's syndrome.⁴⁷

DiGeorge's syndrome is a rare congenital abnormality, resulting when the third and fourth branchial pouches fail to develop normally. A complex of anomalies results, including facial deformities, cardiac malformations, kidney disease, hypoparathyroidism, and an inadequately developed thymus.⁴⁸ The severity of the syndrome varies markedly; the mildest forms require only corrective cardiac surgery, with Vitamin D and calcium supplements to counteract hypoparathyroidism.

Severe forms of the syndrome are characterized by profound immunodeficiency because of the lack of thymic function and resultant lack of T-cell function. It is this aspect of the disease that is amenable to correction by the transplantation of fetal thymus. The results of fetal thymic transplantation indicate that 8 of 26 patients treated by transplant enjoyed extended survival.⁴⁹ Rapid restoration of function has been observed in some patients (within days), while several months are required in other patients. Some success has also been achieved with the use of

cultured fetal thymic cells. Transplantation of tissue rather than extract appears to be required for optimal results in the more severe forms of the disease. DiGeorge's syndrome remains a rare paediatric disease, but it is important as a model of therapy where the transplantation of human fetal cells is the treatment of choice. This success story has generated hope for the effective application of fetal tissue transplantation to a variety of other disorders.

Secondary Applications

Transplantation in Parkinson's Disease

Parkinson's disease is a commonly recognized condition with characteristic signs: tremors, muscular rigidity, and slowness of body movement (bradykinesia). The underlying dysfunction reflects a deterioration of the brain's dopamine-producing substantia nigra cells, cells critical to processes involved in the initiation and control of movement. There is no known pharmacological treatment to halt or reverse the neuronal degeneration. Varying degrees of symptomatic control can be achieved with combinations of drugs, including anticholinergics, propranolol, amantadine, and, most important, L-dopa — a precursor to dopamine. Typically, symptomatic control with various drug protocols fails through the progress of the disease, and the side-effects of drug therapy may be formidable.⁵⁰ More than 70 000 persons in Canada suffer from parkinsonism, and about 5 500 new cases are diagnosed each year. At any point in time it is estimated that nearly 7 000 cases are refractory to conventional drug therapies; not all of these are suitable candidates for fetal tissue transplantation.⁵¹

Experimental parkinsonism has been induced in a number of animal models; although the etiology of Parkinson's in humans is not clearly understood, it is possible to induce the degeneration of substantia nigra cells. This has been accomplished in rats using 6-hydroxydopamine, a chemical analogue of dopamine known to cause selective deterioration of the substantia nigra pathways.⁵² Similarly, a recent experimental model for Parkinson's was developed, quite by accident, in non-human primates. A profound Parkinson syndrome was clinically observed in a number of individuals who had ingested N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a homemade semi-synthetic narcotic. The brain damage suffered by these persons appeared identical to that observed in idiopathic (without a known cause) Parkinson's disease. Further investigations demonstrated that MPTP causes selective degeneration of substantia nigra, and elicits motor symptoms very similar to those seen in Parkinson's patients when administered to non-human primates.⁵³

Both rodent and non-human primate models for Parkinson's are extremely valuable in the development of clinical management approaches to the disease, although these chemical lesion models are far more limited in revealing the etiology of Parkinson's. The non-human primate model is

particularly important in the development of neurosurgical approaches, including the transplantation of fetal neural tissue. Fetal mid-brain material from both animal and human sources has been demonstrated to survive and function when transplanted into the rodent model for Parkinson's disease.⁵⁴ There are limits to the extent to which results from animal models can be generalized to fetal neural transplantation in humans; however, the results of such studies form the basis for considering clinical trials using fetal tissue transplantation in human subjects.

The transplantation of human fetal mid-brain material has been reported in a small number of patients from Britain, Mexico, Sweden, and the United States, almost exclusively in Parkinson's patients. The first published report came from Mexico, where two parkinsonian patients were transplanted with fetal tissue derived from a spontaneously aborted fetus of 13 weeks' gestation.⁵⁵ The report indicated that both patients experienced substantial symptomatic improvement, yet these cases have been strongly criticized on the grounds that fetal neural tissue of 13 weeks' gestation is too mature to survive the transplant procedure and undergo proliferation and functional development in the host.⁵⁶

In Britain, the successful transplantation of fetal substantia nigra in two Parkinson's patients has been reported, and at least 12 patients are claimed to have received a transplant, although details are not available.⁵⁷ In the United States, one case report has come from the University of Colorado, and a randomized controlled clinical trial involving 20 Parkinson's patients is under way at Yale University; both studies are supported by private funds, owing to the National Institutes of Health ban on government funding of transplantation research using electively aborted fetal material.⁵⁸

The most detailed reports of transplantation of fetal substantia nigra material in two patients with Parkinson's disease come from Sweden. Both patients received immuno-suppressive drug therapy; six months after the transplant, only minimal improvement in the patients' subjective experience of the disease was reported. No reduction in conventional medication requirements was seen, although neurophysiological examination indicated small, significant improvements on the side of the body opposite the side of the brain graft. The investigators concluded that the therapy as performed did not effect a significant clinical improvement, but that neurophysiological findings provide a strong rationale to pursue the approach, both in animal studies and in clinical trials.⁵⁹

In Canada, researchers at Dalhousie University and the Victoria General Hospital in Halifax have developed a stringent patient selection protocol for a preliminary clinical fetal tissue transplantation trial that was scheduled to begin in 1991. The following criteria for patient selection have been set: confirmed diagnosis of idiopathic Parkinson's disease by clinical findings and abnormality on positron-emission tomography (PET scan), progressive disease to point of dependence, good initial response to drug

therapy with maximum dosage no longer providing relief or where side-effects are intolerable, and availability for and commitment to regular detailed assessment prior to surgery and for follow-up throughout the post-operative period. Patients are to be selected from the hospital's Movement Disorder Clinic, and technical assistance in fetal tissue handling will be provided by basic researchers at Dalhousie University.

This clinical trial represents a pilot project involving fewer than 10 patients. The president of the hospital emphasizes that ethical and scientific consultations have been conducted across the country, and that the trial involves patients for whom conventional therapy can now offer only a marginal existence.⁶⁰ The results of this carefully planned and public investigation may soon provide much-needed direction concerning the future of fetal neural transplantation as a legitimate therapy for certain end-stage Parkinson's patients.

Transplantation in Type I Diabetes

Diabetes mellitus is a serious and common disease with two major forms — insulin-dependent (juvenile — Type I) and non-insulin dependent (Type II). Both disease states are characterized by persistent elevation of blood sugar; the term diabetes mellitus means "sweet urine."

Type I diabetes accounts for about 10 percent of all diabetes cases. A decrease or complete loss of pancreatic insulin results from the selective destruction of the pancreatic islet cells responsible for the production of insulin. The average age of onset is 12 years, and symptoms include weight loss, increased urination and thirst, and severe fatigue. Acutely, the disease can be fatal if blood ketone levels increase as a result of failed carbohydrate metabolism. Some 15 years after the onset of the disease, many patients acquire secondary complications, including retinopathy (loss of vision), neuropathy (destruction of peripheral nerves), renal disease, cardiovascular disease, severe peripheral vascular disease, and susceptibility to infections. The reason for the loss of pancreatic cells is not known, although an immunological reaction is suspected and a genetic component may be present. The disease has high rates of morbidity and mortality in those afflicted. There is no cure, and patients are treated with daily injections of exogenous insulin.

By contrast, non-insulin dependent (Type II) diabetes has an average age of onset of 40 years and is strongly associated with obesity, with clear heritable tendencies. Retinopathy, renal disease, and cardiovascular disease are long-term complications. Weight control and oral medication can control the disease in most patients; only a few require insulin to obtain normal blood sugar levels.⁶¹

Pancreatic transplantation from cadaveric and living related donors has enjoyed some success in the treatment of Type I diabetes: worldwide, 1 549 pancreas transplants were performed in 1 440 patients between December 1966 and June 1988. A one-year graft function was observed in 49 percent of patients, with a survival rate of 85 percent. Unfortunately,

these results decline rapidly following the first year after the transplant. Significant immuno-suppressive regimens are often required, and the long-term use of these drugs contributes significantly to major organ failure.⁶² More recently, investigators have considered the use of pancreatic tissue from aborted fetuses as a more attractive source of transplant material. The growth potential of the tissue, its possible reduced immunogenicity, and the consistently available supply suggest important therapeutic potential.

The development and functioning of the human pancreas *in utero* take place between the eighth and twentieth weeks of gestation. Islet cells can be observed in the eighth week, alpha (glucagon-producing) cells at nine weeks, delta cells (somatostatin) in the tenth week, and beta (insulin-producing) cells at 11 weeks, with further growth and differentiation of minor cell types after 11 weeks. Great growth potential is exhibited by fetal islet cells: insulin content increases from 2 units/gram at 11 to 13 weeks' gestation to 6 units/gram at 23 to 24 weeks.⁶³ The ability of developing fetal pancreatic islet tissue to respond physiologically to glucose by secreting insulin does not occur until late gestation, maturing over a period of months. Frozen fetal pancreatic tissue can be stored successfully using DMSO (70 percent survival) and retains its endocrine function and histological characteristics on thawing.⁶⁴

Animal studies have been used to investigate the effectiveness of fetal pancreatic transplantation in rodent models of diabetes. The transplant of one fetal rat pancreas may be sufficient to normalize blood sugar, ameliorate frequent thirst and urination, and assist weight gain in the diabetic rat. Several fetal pancreatic transplants are required to achieve overall normalization of carbohydrate metabolism, with reversal of secondary complications possible.⁶⁵ The limitations of transplantation from cadaveric and living related donors and the results of animal studies form the basis for clinical experimentation in fetal pancreatic transplant for human diabetic patients.

The first report of human fetal pancreatic transplantation appeared in 1938; no demonstrable effect was seen in two patients.⁶⁶ Possibly 600 Type I diabetes patients have received fetal pancreatic transplant worldwide; results reported in the International Registry have been disappointing. Most recipients received pancreas from fetuses at 16 to 20 weeks' gestation.⁶⁷ One study followed five patients for one year; insulin-producing cells were recovered from the patients at 9 to 14 months after transplant, although histological evidence of rejection was noted.⁶⁸

Clinical transplantation research in this area was premised on the notion that fetal pancreatic tissue would prove less immunogenic than that from adult sources. Evidence is mounting that fetal pancreatic tissue may be at least as immunogenic as adult tissue.⁶⁹ The clinical failure of fetal pancreatic transplants when compared with animal studies is not understood: immunogenicity may be responsible, or technical considerations in the handling and processing of the fetal pancreatic cells may be involved.

Further basic research is needed to assess whether fetal pancreatic tissue transplant holds any real promise in the treatment of juvenile diabetes, particularly when compared with promising alternative research in the treatment of diabetes.

Tertiary (Basic) Applications

Human fetal tissues have been used or considered for use in a variety of clinical conditions. This part of the paper outlines these uses and reports the results of fetal therapy where known. It is important to note that these applications are generally primitive in terms of our experience and knowledge of their future potential.

Severe Combined Immune Deficiency (SCID)

SCID encompasses a number of rare congenital disorders where gene defects result in the failure of one or both lymphocyte lines (T or B) to function. Children born without lymphocyte function usually die as a result of infection before their first birthday. SCID became more widely known as a result of the press coverage of the "boy in the bubble," who lived in complete sterile isolation for most of his life. These diseases may show increased frequency in identifiable ethnic groups.⁷⁰

Complete cure for this condition can be effected by HLA (tissue-typing) identical bone marrow transplant, and this is the treatment of choice. The result of the transplant is the appearance of a T-cell population carrying the donor's genetic markers. This therapy is limited by the fact that related HLA-matched donors cannot be found for more than 60 percent of patients.⁷¹ In such cases the transplant of allogeneic human fetal liver, with or without fetal thymus, may be attempted; one report claimed survival of 6 out of 11 patients transplanted with liver and thymus tissues from the same fetus. No tissue matching was attempted in this study. A summary of 64 SCID patients who received fetal liver transplantation at various centres indicates that 22 patients had durable engraftments.⁷² Thus fetal liver transplantation with or without fetal thymus may prove an effective treatment alternative in SCID where HLA-matched bone marrow is not available for transplant.

Leukemia

The term leukemia refers to a number of malignancies of the various types of white blood cells. Four major classes are identified: ALL (acute lymphocytic leukemia), AML (acute myelogenous leukemia), CLL (chronic lymphocytic leukemia), and CML (chronic myelogenous leukemia). The incidence of these forms varies with age, with ALL most common in children and young adults. AML is the leading cause of cancer deaths in children.⁷³

Transplantation of bone marrow after destruction of the patient's own bone marrow by radiation and chemotherapy has greatly improved cure rates. Where HLA-matched or partly matched donors have been

unavailable, fetal liver has been transplanted in a small number of leukemia patients. The results are difficult to interpret, because the numbers are small, patients varied widely in severity of illness, and the criteria used to establish engraftment vary from centre to centre.⁷⁴ It is believed that recovery of the patient's own bone marrow occurs earlier after chemotherapy and fetal liver transplantation than with chemotherapy alone. Further basic investigation is warranted to elucidate the mechanisms of this recovery and to assess the future of fetal liver transplantation for certain leukemic patients.

Aplastic Anaemia

This term refers to a variety of disease states characterized by the failure of the bone marrow to produce normal amounts of formed blood elements — red cells, white cells, and platelets. Approximately 50 percent of cases are idiopathic, with the balance attributed to agents such as viruses, chemical exposure, drug side-effects, and radiation accidents. Untreated, 50 percent of patients die within one year of diagnosis. Immuno-suppressive therapy with steroids, treatment with immuno-globulin, and HLA-identical bone marrow transplant are therapies.

Human fetal liver was transplanted into 122 patients with aplastic anaemia between 1960 and 1986, and 66 of these patients experienced partial or complete recovery. The mechanism of the recovery is not understood, as tissue engraftment was poor (much poorer than that seen when fetal liver is transplanted in leukemia patients).⁷⁵ Controlled randomized clinical trials would yield much-needed data about the efficacy of fetal liver transplantation in treating aplastic anaemia, and basic laboratory research is needed to reveal the mechanism of its success.

Inherited Metabolic Storage Disorders

Such disorders are one type of a broad classification of diseases labelled inherited metabolic diseases, where the principal defect is an absent or abnormal gene failing to produce a required enzyme. The absence of the enzyme results in disruption of function at both cellular and organ levels. Such storage disorders include Tay-Sachs disease and a rapidly growing list of disorders newly identified by molecular probes. The defect in metabolism prevents normal breakdown and elimination of biochemical substances, and the accumulation of materials at the cellular level may result in skeletal deformation, physical and intellectual retardation, and neurological abnormalities leading to severe dysfunction and death.⁷⁶

A range of therapies for the management of metabolic disorders exists, but they are generally very limited in effectiveness. A report of 21 patients transplanted with human fetal liver cells indicates partial and transient benefits, with stabilization of tissue deposits, although few data are available.⁷⁷ The use of transplanted fetal material remains an area of basic research into the treatment of these rare diseases.

Radiation Poisoning

Radiation poisoning from thermonuclear bombs, nuclear power plant accidents, and medical diagnostic and treatment accidents produces acute and long-term health effects. Bone marrow in particular is exquisitely sensitive to damage by radiation; bone marrow suppression is one of the earliest acute effects of exposure to radiation, even at relatively low doses. Leukemias are a common long-term complication of radiation exposure.

Both bone marrow transplantation and fetal liver transplantation were used to treat victims of the Chernobyl accident. The effectiveness of the treatment is difficult to evaluate at this time, owing to limited numbers and follow-up time and wide discrepancies in patient age, health status, and severity of illness. The use of fetal cells may yet prove important in the treatment of this peculiarly modern menace.⁷⁸

Feto-Fetal Therapy

Two animal models for the *in utero* transplantation of fetal haematopoietic cells have been developed. Diseases that may be amenable to treatment by this method include beta-thalassemia, sickle cell anaemia, Wiskott-Aldrich syndrome, chronic granulomatous disease, Kostman's syndrome, infantile malignant osteopetrosis, Chédiak-Higashi syndrome, Maroteaux-Lamy syndrome, and SCID.⁷⁹ The method involves the transplantation of fetal haematopoietic stem cells to fetal rhesus monkeys and fetal sheep *in utero*. The preliminary results of these studies indicate successful tissue engraftment and freedom from graft versus host disease for up to two years.⁸⁰ Such investigations point to yet another application of fetal liver cells in transplantation therapy for disease in humans.

Alzheimer's Disease

Alzheimer's disease is an incurable progressive condition resulting in increasing loss of higher cognitive functions. The cause of Alzheimer's is not known; however, clinical findings are well defined, and a complement of neurological pathologies are consistently noted at post-mortem examination of the brain tissue of Alzheimer's patients. These neuropathologies include neurofibrillary tangles and plaque formations in cholinergic neurons, increased cellular aluminum content associated with these formations, and diffuse neuronal degeneration.

While the cause of Alzheimer's is not known, certain pathological features of the disease have led some researchers to suggest a common etiology with Parkinson's disease.⁸¹ In recent investigations using a marmoset monkey model, memory loss for learned tasks was induced by surgical lesion of cholinergic neurons. The animals were then treated by surgical grafting of embryonic cholinergic tissue to the forebrain. The ability to perform the previously learned tasks was restored. The authors are concerned about generalizing the findings to Alzheimer's patients, however, since the brain degeneration associated with this disease is quite

diffuse.⁸² This is in contrast to the relatively discrete lesion seen in Parkinson's disease.

The application of fetal cholinergic transplantation in humans with Alzheimer's remains some years away; the societal importance of this disease and the present lack of effective treatments suggest that fetal tissue transplantation will be given serious consideration as a therapeutic possibility.

Acquired Immunodeficiency Syndrome (AIDS)

AIDS reached pandemic proportions in the 1980s. The HIV was demonstrated to be the etiologic agent in 1984, yet there is neither cure nor vaccine. Certain drugs may prolong immune function in some patients, but AIDS is a uniformly fatal disease. Fetal cells have been used in the culture and manipulation of the HIV in culture, and there is some evidence that fetal hepatic cells may suppress the virus. Such research remains at the most basic level of investigation; yet it is certain that fetal cells will continue to be used for study of the HIV, and the use of fetal cells for AIDS therapy is a possibility.⁸³

Plastic Surgery

Quite recently, fetal connective tissue and cartilage have been studied in a pig model of mini-autograft dermal injections. The tissue was compared with the conventional materials collagen and silicone. The results indicate that the fetal tissue should not be recommended for soft tissue filling in the face, because of localized inflammatory reaction. The report did, however, recommend research into the processing of collagen from fetal connective tissue for further investigation in this area.⁸⁴

It is likely that other potential applications of fetal tissues in the treatment of disease and in industry will arise, as understanding of the remarkable properties of fetal tissues increases.

Alternatives and Limitations: Embryo Research

This segment of the paper briefly highlights major limitations and possible alternatives to the use of human embryos for research.

Perhaps the single most important factor limiting the use of human embryos in research, particularly basic research, is the relative scarcity of access to early pre-embryos. Human embryos created *in vitro* are created almost exclusively for the purpose of transfer to the uterus, in the hope of attaining a successful pregnancy. This is also true of the few instances where uterine lavage can be used for preimplantation genetic diagnosis. Primary embryo research (the clinical trial) has been noted as a broad category of human pre-embryo research; this is the research that takes place in infertility treatment programs offering services such as *in vitro* fertilization (IVF), GIFT, ZIFT, PROST, and embryo cryopreservation.

Secondary embryo research comprises those clinical experiments where no precedent human observations or only minimal clinical data form the basis of the intervention, yet a clear possibility of important clinical benefit is discernible.⁸⁵

Clinical research in both the primary and the secondary categories circumscribes a peculiar area of biomedical endeavour that overlaps both treatment and investigation. It is likely that both categories will continue to be active areas of human pre-embryo research, given the demand for improved clinical results with the new assisted reproductive technologies. Some have argued that when the embryo is regarded as the sole research subject, the woman undergoing treatment for infertility may be lost sight of. Further arguments have been advanced that research that is intended to be therapeutic for the embryo may be non-therapeutic for the woman. Critics of this type of research argue that regulation of embryo research is necessary and that it must take account of the relationship between a woman's treatment, the dependence of embryos on women for gestation, and "the empirical uncertain nature of biomedical knowledge."⁸⁶ Regulation of pre-embryo research is being implemented in a growing number of jurisdictions. It may prove an important limiting factor in future embryo research.

The third category of human pre-embryo research is pre-clinical basic research that cannot be pursued adequately by the use of animal models; preimplantation genetic diagnosis is one example. It is distinguished from the first two categories in that there is a declared intent from the outset that the experimental subjects will not be transferred into a receptive uterus.

Animal models provide the starting point for such investigations and should be exploited aggressively. However, there is ample evidence of limitations of generalizing animal model results to the human embryo. For example, there has been great interest in developing techniques for the cryopreservation of human oocytes as an alternative to embryo freezing, in part to circumvent ethical, social, and legal concerns about embryo freezing. It is now possible to freeze, thaw, fertilize, and transfer mouse ova, with normal resultant offspring. Investigations into the freezing of human ova have been far less successful, owing to subtle but critical structural differences between ova from the two species. Therefore, freezing of embryos or zygotes is the preferred procedure at this time.⁸⁷

Thus, while animal models are essential for preliminary basic embryo research, there are strong procedural reasons for tertiary human embryo research in order to safeguard offspring and parents. Again, the major limiting factor to such research continues to be the scarcity of human embryos for any purpose other than attempting pregnancy.

Alternatives and Limitations: Fetal Tissue Research

Currently, there appears to be more than sufficient fetal tissue available for research and therapy from elective abortions alone. In the United States 1.6 million legal elective abortions are performed annually, and approximately 85 000 are performed in Canada. More than 90 percent of these pregnancies are terminated in the first trimester.⁸⁸ Most research applications require fetal tissues between 8 and 14 weeks' gestation, the exception being fetal pancreatic transplantation for juvenile diabetes.

Limitations to this apparently adequate supply are real, however. There is no accepted consent and screening process in place for obtaining aborted fetal material from women undergoing abortion. Concerns have been voiced about how consent could be obtained in a non-coercive fashion and about whether the woman who undergoes abortion has a moral right to donate the fetus. The current method of choice for first-trimester abortion is vacuum aspiration; this method severely macerates the collected conceptus material, so that distinct cell types are recognizable in only about 10 percent of cases. Alternative methods of abortion (D&C) yield improved fetal specimen quality but pose an increased morbidity risk for the woman.

Perhaps in competition with a perceived need for fetal tissue for research and treatment is current research directed at new, earlier, and safer methods of first-trimester abortion — menstrual extraction and the abortifacient pill. Refinement and implementation of these or other early abortion methods would severely compromise the amount of fetal tissue available for research. In addition, there is debate about whether the use of aborted fetal tissue for research is ethically permissible, and this may sully the acceptability of using fetal tissues from elective abortions.⁸⁹

These concerns have rekindled interest in the use of fetal material from spontaneous abortions. The potential problems of timing, tissue necrosis, genetic defect, and infection have been outlined; nonetheless, investigation continues into the potential exploitation of this source.⁹⁰ Non-surgical treatment of ectopic pregnancy is likely to eliminate this as a potential source of fetal tissues.⁹¹

A number of investigations are currently under way to examine the use of animal fetal tissue for transplantation and therapy in humans (xenografts). In the early 1960s, grafting of baboon and chimpanzee kidneys was attempted, as were liver grafts from chimpanzees. Four attempts have been made to graft animal hearts to humans. The results of these clinical experiments have been extremely poor, and the experiments have been largely abandoned, owing to the severe graft rejection that results. The relatively non-immunogenic fetal tissue of non-human primates may prove more successful in transplantation experimentation.⁹² The use of non-human primate fetal tissues for therapy will undoubtedly raise a host of ethical and social controversies in its own right.

A Schematic Overview: Embryo and Fetal Tissue Research

Social, Ethical, and Legal Issues

Primary
Research

Assisted Reproductive Technologies,
Quality Control,
Male Factor Research

Industry, Viral
Research, DiGeorge's
Syndrome

Secondary
Research

Embryo-Derived
Factors, Implantation
Biology

Transplantation in
Parkinson's Disease
and Type I Diabetes

Tertiary
Research

Pre-Implantation
Diagnosis, Screening,
Sex Selection, Gene Therapy

SCID, Leukemia,
Aplastic Anaemia,
Radiation Poisoning,
Feto-Fetal Therapy, Alzheimer's,
AIDS, Cosmetic Surgery

Limitations

Supply
Ethical Concerns
Legislation and Regulation
Public Policy Formation

Some research has shown promise that fetal cells may be successfully cultured and developed as continuing cell lines, thus ensuring a supply of tissues for both basic cell research and transplant. Cryopreservation of fetal tissue is successful for some cell types.⁹³ The refinement of these methodologies may serve in the future to address many of the practical, ethical, and social issues surrounding the use of fetal tissues.

In policy terms, the use of fetal tissues for treatment ideally does not detract from the basic research tasks of determining the underlying pathology responsible for diseases such as Parkinson's, diabetes, Alzheimer's, and the inherited disorders, and of discovering cures for these diseases. Today, Canada has no public policy governing the use of fetal tissues. In view of the rapid proliferation of applications for fetal tissues in industry and biomedicine, the need for public policy development is pressing.⁹⁴

Glossary of Terms

Allogeneic: cells and tissues from the same species.

Blastomere: the distinct, individual cells of the early embryo.

Chorionic villus: the projections of the outermost coating or layer encasing the developing embryo or fetus.

Cleavage: the division of a given cell, giving rise to two distinct cells.

Cryopreservation: the long-term preservation of organisms by specialized freezing and thawing techniques.

Cytogenetics: the study of genetics at the cellular level.

Cytosolic leakage: leakage or spill of cellular contents resulting from disruption of the cell membrane.

Dopamine: a biochemical messenger released by substantia nigra cells; important for normal initiation and control of movement.

Fetal cardiac arrhythmias in utero: abnormal fetal heart rates, detected before the fetus is born.

Haematopoietic cells: those cells involved in production of blood cells and blood components.

Human chorionic gonadotropin: also hcg, a hormone of early pregnancy; testing for hcg in maternal blood and urine is a routine diagnostic test for pregnancy.

Human germ cells: the human reproductive cells, namely sperm and egg cells, and the progenitor cells giving rise to them.

Hypoparathyroidism: congenital or acquired, failure or absence of the parathyroid glands; linked to failed development of the thymus in DiGeorge's syndrome.

Immunogenic: provoking an immune response.

Immunogenicity: the tendency or degree to which any material (including biological materials) will provoke an immune response in a recipient of that material.

Intrauterine fetal surgery: surgical procedures performed on a fetus while it remains in the maternal environment.

Non-exogenous ovarian stimulation cycle: ovarian cycle where the natural follicular development is neither induced nor enhanced by treatment with hormones or drugs (normal cycle).

Polar body: a cellular extrusion resulting from the last meiotic (final maturation) division of the oocyte.

Polypliody: appearance of more than two pronuclear bodies in a newly fertilized ovum; see polyspermy.

Polyspermy: impregnation of an ovum by more than one sperm cell. When this occurs, more than two pronuclei can be seen by microscopic visualization in the early hours following fertilization.

Pronuclei: the separate nuclei of the sperm and ovum, before these unite to form the single definitive nucleus of the fertilized ovum.

Somatic cells: the cells of the human body, excluding the germ cells (ova and spermatozoa).

Substantia nigra cells: literally, cells of the "black substance"; these brain cells produce dopamine and are implicated in Parkinson's disease. The name reflects their appearance.

Teratogenicity: that quality of any agent — chemical, biological, or physical — that contributes to or causes malformation of a developing organism.

Totipotentiality: the capacity of a single cell to develop into or to generate a complete organism.

Twining procedures: technical processes used to divide a mammalian embryo in its early stages (usually four or eight cells), so that two identical embryos result; if these are successfully transferred to a receptive uterus, identical offspring may result. This effect also occurs naturally, if rarely, in humans.

Zona pellucida: the transparent membrane forming the cell wall in the mammalian ovum.

Zygote: the organism resulting from the union of the human germ cells — ovum and the sperm — with its new and distinct genetic constitution.

Notes

1. C.R. Austin, "Fertilization," in *Germ Cells and Fertilization*, ed. C.R. Austin and R.V. Short (Cambridge: Cambridge University Press, 1972), 103-23.
2. C.J. Roberts and C.R. Lowe, "Where Have All the Conceptions Gone?" *Lancet* (1 March 1975): 498-99.
3. American Fertility Society, Ethics Committee, "The Biologic Characteristics of the Preembryo," *Fertility and Sterility* 53 (Suppl. 2)(1990): 31S-33S.
4. M. Monk, "A Stem-Line Model for Cellular and Chromosomal Differentiation in Early Mouse Development," *Differentiation* 19 (1981): 71-76.
5. N. Le Douarin and A. McLaren, eds., *Chimeras in Developmental Biology* (London: Academic Press, 1984).
6. M.H. Kaufman, "The Origin, Properties and Fate of Trophoblast in the Mouse," in *Biology of Trophoblast*, ed. Y.W. Loke and A. Whyte (New York: Elsevier Science, 1983), 23-68.

7. M. Collier et al., "The Production of Embryo Derived Platelet Activating Factor by Human Embryos and Its Relationship to Pregnancy Outcome," *Clinical Reproduction and Fertility* 5 (1987): 307-308; N.R. Spinks et al., "Embryo Derived Platelet Activating Factor: A Mediator for the Establishment of Pregnancy in the Mouse," *Clinical Reproduction and Fertility* 5 (1987): 308-309.
8. T.M. Crombleholme et al., "Transplantation of Fetal Cells," *American Journal of Obstetrics and Gynecology* 164 (1991): 218-30.
9. M.Z. Ratajczak, "Experimental Aspects of Transplantation of Haemopoietic Cells of Fetal Liver," *Archivum Immunologiae et Therapiae Experimentalis* 36 (1988): 235-43.
10. Y.S. Mullen et al., "Complete Reversal of Experimental Diabetes Mellitus in Rats by a Single Fetal Pancreas," *Science* 195 (1977): 68-70; J.R. Sladek et al., "Reversal of Parkinsonism by Fetal Nerve Cell Transplants in Primate Brain," *Annals of the New York Academy of Sciences* 495 (1987): 641-57.
11. B. Gustavii, "Fetal Brain Transplantation for Parkinson's Disease: Technique for Obtaining Donor Tissue," *Lancet* (11 March 1989): 565.
12. B.G. Benoit and J.D. Grimes, "Technical Considerations in Brain Grafting for Parkinson's Disease," *Transplantation/Implantation Today* 5 (November 1988): 59-68.
13. R. Auerbach, "Qualities of Fetal Cells and Tissues," in *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2 (Bethesda: National Institutes of Health, 1988), D28-D31.
14. D. Jones, "Hospital's Decision to Pursue Fetal Transplantation Upsets Antiabortionists," *Canadian Medical Association Journal* 142 (1990): 1274-77.
15. K.H. Thanki and C.L. Schmidt, "Follicular Development and Oocyte Maturation After Stimulation with Gonadotropins Versus Leuprolide Acetate/Gonadotropins During In Vitro Fertilization," *Fertility and Sterility* 54 (1990): 656-60.
16. American Fertility Society, "Minimal Standards for Programs of In Vitro Fertilization," *Fertility and Sterility* 41 (1984): 13.
17. L. Formigli et al., "Non-Surgical Flushing of the Uterus for Pre-Embryo Recovery: Possible Clinical Applications," *Human Reproduction* 5 (1990): 329-35.
18. Canadian Fertility and Andrology Society and Society of Obstetricians and Gynaecologists of Canada, *Ethical Considerations of the New Reproductive Technologies* (Toronto: Ribosome Communications, 1990), 22.
19. G. Wright et al., "Observations on the Morphology of Pronuclei and Nucleoli in Human Zygotes and Implications for Cryopreservation," *Human Reproduction* 5 (1990): 109-15.
20. C. Tietze and S.K. Henshaw, "Incidence of Abortion," in *Induced Abortion: A World Review 1986*, 6th ed. (New York: Alan Guttmacher Institute, 1986), 30-41.
21. S.K. Henshaw et al., "A Portrait of American Women Who Obtain Abortions," *Family Planning Perspectives* 17 (March/April 1985): 90-96.
22. H. Kalter, "Diabetes and Spontaneous Abortion: A Historical Review," *American Journal of Obstetrics and Gynecology* 156 (1987): 1243-53; H.J. Huisjes,

"Spontaneous Abortion," *Current Reviews in Obstetrics and Gynecology*, No. 8 (Edinburgh: Churchill Livingstone, 1984), 34.

23. H. Fernandez et al., "Spontaneous Resolution of Ectopic Pregnancy," *Obstetrics and Gynecology* 71 (1988): 171-74.

24. M. Pansky, "Local Methotrexate Injection: A Non-Surgical Treatment of Ectopic Pregnancy," *American Journal of Obstetrics and Gynecology* 161 (1989): 393-96.

25. P. Braude and M. Johnson, "Embryo Research: Yes or No?" *British Medical Journal* (2 December 1989): 1349-50; D.H. Smith et al., "Zygote Intra-Fallopian Transfer: The Last Word or the Worst Choice?" *Clinical Reproduction and Fertility* 5 (1987): 400-401.

26. P. Quinn et al., "Culture Factors Affecting the Success Rate of In Vitro Fertilization and Embryo Transfer," *Annals of the New York Academy of Sciences* 442 (1985): 195-204.

27. H.H. Sachs and M.M. Quigley, "Culture Media for In Vitro Fertilization," *Fertility and Sterility* 53 (1990): 953.

28. M.M. Seibel, "A New Era in Reproductive Technology: IVF, GIFT, and Donated Embryos and Gametes," *New England Journal of Medicine* 318 (1988): 828-34; A. Trounson and L. Freemann, "Role of Cryopreservation of Human Oocytes and Embryos in an IVF Program," in *Progress in Infertility*, 3d ed., ed. S.J. Behrman, R.W. Kistner, and G.W. Patton (Boston: Little, Brown, 1988), 621-29.

29. Y. DuPlessis et al., "A Comparison of Implantation Rates Between Fresh and Frozen-Thawed Embryo Replacement Cycles," in *Proceedings of the Seventh Annual Scientific Meeting of the Fertility Society of Australia* (Newcastle: Fertility Society of Australia, 1988).

30. D. Levran et al., "Pregnancy Potential of Human Oocytes — The Effect of Cryopreservation," *New England Journal of Medicine* 323 (1990): 1153-56.

31. S. Gordts et al., "Survival and Pregnancy Outcome After Ultrarapid Freezing of Human Embryos," *Fertility and Sterility* 53 (1990): 469-72.

32. M.A. Mullen, H.W.G. Baker, and W.I.H. Johnston, "A Clinical Trial Utilizing Nycodenzo Discontinuous Gradient Preparation for In Vitro Fertilisation," *Clinical Reproduction and Fertility* 5 (1987): 289-90; H.E. Malter and J. Cohen, "Blastocyst Formation and Hatching In Vitro Following Zona Drilling of Mouse and Human Embryos," *Gamete Research* 24 (1989): 67-80.

33. G. Vines, "Why Experiment on Human Embryos?" *New Scientist* 124 (4 November 1989): 48-50.

34. J. Cherfas, "Britain's Lords Debate Embryo Research," *Science* 246 (1989): 1554-55.

35. V.N. Bolton et al., "Development of Spare Human Preimplantation Embryos In Vitro: An Analysis of the Correlations Among Gross Morphology, Cleavage Rates, and Development to the Blastocyst," *Journal of In Vitro Fertilization and Embryo Transfer* 6 (1989): 30-35.

36. R.J. Paulson, M.V. Sauer, and R.A. Lobo, "Embryo Implantation After Human In Vitro Fertilization: Importance of Endometrial Receptivity," *Fertility and Sterility* 53 (1990): 870-74.

37. G. Vines, "New Insights into Early Embryos," *New Scientist* 115 (9 July 1987): 22-23.
38. B. Brambati and L. Tului, "Preimplantation Genetic Diagnosis: A New Simple Uterine Washing System," *Human Reproduction* 5 (1990): 448-50.
39. M. Michael and S. Buckle, "Screening for Genetic Disorders: Therapeutic Abortion and IVF," *Journal of Medical Ethics* 16 (1990): 43-47.
40. J.R. Riordan et al., "Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA," *Science* 245 (1989): 1066-73.
41. M. Monk, "Embryo Research and Genetic Disease," *New Scientist* 125 (6 January 1990): 56-57.
42. G. McBride, "Combo Technology Checks Genes of Preimplanted Embryo," *Medical Post* (5 March 1991), p. 21.
43. T. Friedmann, "Progress Toward Human Gene Therapy," *Science* 244 (1989): 1275-81; K. Cornetta, R. Wieder, and W.F. Anderson, "Gene Transfer into Primates and Prospects for Gene Therapy in Humans," *Progress in Nucleic Acid Research and Molecular Biology* 36 (1989): 311-22.
44. P.A. Baird, "Gene Therapy," *Lancet* (22 April 1989): 902.
45. J.T. Hansen and J.R. Sladek, "Fetal Research," *Science* 246 (1989): 775-79.
46. P.H. Phipps et al., "Rapid Detection of Influenza Virus Infections in Human Fetal Lung Diploid Cell Cultures," *Journal of Infection* 18 (1989): 269-78; T. Ochiya et al., "An *In Vitro* System for Infection with Hepatitis B Virus that Uses Primary Fetal Hepatocytes," *Proceedings of the National Academy of Sciences of the United States of America* 86 (1989): 1875-79; K. Numazaki et al., "Replication of Measles Virus in Cultured Human Thymic Epithelial Cells," *Journal of Medical Virology* 27 (1989): 52-58.
47. W.W. Cleveland et al., "Foetal Thymic Transplant in a Case of DiGeorge's Syndrome," *Lancet* (7 December 1968): 1211-14; C.S. August et al., "Implantation of a Foetal Thymus, Restoring Immunological Competence in a Patient with Thymic Aplasia (DiGeorge's Syndrome)," *Lancet* (7 December 1968): 1210-11.
48. M.D. Cooper, R.D.A. Peterson, and R.A. Good, "A New Concept of the Cellular Basis of Immunity," *Journal of Pediatrics* 67 (1965): 907-908; D.J. Barrett et al., "Clinical and Immunologic Spectrum of the DiGeorge Syndrome," *Journal of Clinical and Laboratory Immunology* 6 (1981): 1-6.
49. A.B. Goldsobel, A. Haas, and E.R. Stiehm, "Bone Marrow Transplantation in DiGeorge Syndrome," *Journal of Pediatrics* 111 (1987): 40-44.
50. R.C. Duvoisin, *Parkinson's Disease: A Guide for Patient and Family*, 2d ed. (New York: Raven Press, 1984), 28-57; J.D. Wilson et al., eds., *Harrison's Principles of Internal Medicine: Companion Handbook*, 12th ed. (New York: McGraw-Hill, 1991), 2065-69.
51. A. Rajput, Parkinson's Foundation of Canada Epidemiologist, Dept. of Neurology, University Hospital, Saskatoon, Saskatchewan, telephone conversation with author, January 1991.
52. M.J. Perlow, "Brain Grafting as a Treatment for Parkinson's Disease," *Neurosurgery* 20 (1987): 335-41.

53. R.S. Burns et al., "A Primate Model of Parkinsonism: Selective Destruction of Dopaminergic Neurons in the Pars Compacta of the Substantia Nigra by N-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine," *Proceedings of the National Academy of Sciences of the United States of America* 80 (1983): 4546-50.
54. P. Brundin et al., "Intracerebral Grafting of Dopamine Neurons: Experimental Basis for Clinical Trials in Patients with Parkinson's Disease," *Annals of the New York Academy of Sciences* 495 (1987): 473-96; P. Brundin et al., "Can Human Fetal Dopamine Neuron Grafts Provide a Therapy for Parkinson's Disease?" *Progress in Brain Research* 78 (1988): 441-48.
55. I. Madrazo et al., "Transplantation of Fetal Substantia Nigra and Adrenal Medulla to the Caudate Nucleus in Two Patients with Parkinson's Disease," *New England Journal of Medicine* 318 (1988): 51.
56. C.R. Freed, "Regarding Transplantation of Fetal Substantia Nigra and Adrenal Medulla to the Caudate Nucleus in Two Patients with Parkinson's Disease," *New England Journal of Medicine* 319 (1988): 370.
57. E.R. Hitchcock et al., "Embryos and Parkinson's Disease," *Lancet* (4 June 1988): 1274; R.A.E. Bakay and D.L. Barrow, "Neural Transplantation for Parkinson's Disease," *Journal of Neurosurgery* 69 (1988): 807-10.
58. S. Blakeslee, "In Careful Test, Parkinson's Patient Shows Gains After Fetal-Cell Implant," *New York Times* (2 May 1989), sec. C, p. 3.
59. O. Lindvall et al., "Human Fetal Dopamine Neurons Grafted into the Striatum in Two Patients with Severe Parkinson's Disease: A Detailed Account of Methodology and a 6-Month Follow-Up," *Archives of Neurology* 46 (1989): 615-31.
60. S. Weber, "Fetal Cell Transplant for the Treatment of Parkinson's Disease Scheduled Later in '91," *Medical Post* (15 January 1991), sec. 2, p. 61.
61. Wilson, *Harrison's Principles*, 1739-59.
62. D.E.R. Sutherland and K.C. Moudry, "Pancreas Transplant Registry Report — 1986," *Clinical Transplantation* 1 (1987): 3-17; D.E.R. Sutherland and K.C. Moudry, "Pancreas Transplantation Registry Report," *Transplantation Proceedings* 1 (1989): 2759-62.
63. M. Laitio, R. Lev, and D. Orlic, "The Developing Human Fetal Pancreas: An Ultrastructural and Histochemical Study with Special Reference to Exocrine Cells," *Journal of Anatomy* 117 (1974): 619-34; K.F. Wellmann, B.W. Volk, and P. Brancato, "Ultrastructure and Insulin Content of the Endocrine Pancreas in the Human Fetus," *Laboratory Investigation* 25 (August 1971): 97-103.
64. J. Brown et al., "Cryopreservation of Human Fetal Pancreas," *Diabetes* 29 (Suppl. 1)(1980): 70-73.
65. O.D. Hegre, "Islet Cell Transplantation," in *The Diabetic Pancreas*, 2d ed., ed. B.W. Volk and E.R. Arquila (New York: Plenum Medical Book, 1985), 513-42.
66. H.B. Stone et al., "Further Reports on Grafting of Endocrine Glands," *Mississippi Doctor* (1938): 6-9.
67. M.D. Stegall, D.E.R. Sutherland, and M.A. Hardy, "Registry Report on Clinical Experience with Islet Transplantation," in *Transplantation of the Endocrine Pancreas in Diabetes Mellitus*, ed. R. Van Schilfgaarde and M.A. Hardy (New York: Elsevier Science, 1988), 224-33.

68. B.E. Tuch et al., "Recovery of Human Fetal Pancreas After One Year of Implantation in the Diabetic Patient," *Transplantation* 46 (1988): 865-70.
69. D.A. Hullett et al., "Human Fetal Pancreas — A Potential Source for Transplantation," *Transplantation* 43 (1987): 18-22.
70. J.F. Soothill, A.R. Hayward, and C.B.S. Wood, eds., *Paediatric Immunology* (Oxford: Blackwell Scientific, 1983), 156-211.
71. J.L. Touraine et al., "Fetal Tissue Transplantation, Bone Marrow Transplantation and Prospective Gene Therapy in Severe Immunodeficiencies and Enzyme Deficiencies," *Thymus* 10 (1987): 75-87.
72. R.J. O'Reilly et al., "A Comparative Review of the Results of Transplants of Fully Allogeneic Fetal Liver and HLA-Haplotype Mismatched, T-Cell Depleted Marrow in the Treatment of Severe Combined Immunodeficiency," in *Fetal Liver Transplantation: Proceedings of an International Symposium Held in Pesaro, Italy, September 29-October 1, 1984*, ed. R.P. Gale, J.L. Touraine, and G. Lucarelli (New York: Liss, 1985), 327-42.
73. Wilson, *Harrison's Principles*, 1552-96.
74. R.P. Gale, "Fetal Liver Transplantation in Aplastic Anemia and Leukemia," *Thymus* 10 (1987): 89-94; V. Kochupillai et al., "Fetal Liver Infusion in Acute Myelogenous Leukaemia," *Thymus* 10 (1987): 117-24.
75. V. Kochupillai et al., "Fetal Liver Infusion in Aplastic Anaemia," *Thymus* 10 (1987): 95-102.
76. C.R. Scriver et al., eds., *The Metabolic Basis of Inherited Disease*, 6th ed., 2 vols. (New York: McGraw-Hill, 1989).
77. Touraine, "Fetal Tissue Transplantation."
78. R.P. Gale and Y. Reisner, "The Role of Bone-Marrow Transplants After Nuclear Accidents," *Lancet* (23 April 1988): 923-26.
79. M.R. Harrison et al., "In-Utero Transplantation of Fetal Liver Haemopoietic Stem Cells in Monkeys," *Lancet* (16 December 1989): 1425-27.
80. Crombleholme, "Transplantation of Fetal Cells."
81. R. McGuire, "Parkinson's, Alzheimer's May Share Common Etiology," *Medical Post* (16 October 1990), p. 31.
82. American Fertility Society, Ethics Committee, "Memory Loss Reversible with Brain Tissue Transplant," *Canadian Doctor* 56 (November 1990): 2; A. Fine, "Transplantation in the Central Nervous System," *Scientific American* 255 (August 1986): 52-58B.
83. C. McGourty, "Ban on Use of Fetal Tissue to Continue," *Nature* 342 (1989): 105.
84. U.T. Hinderer and J. Escalona, "Dermal and Subdermal Tissue Filling with Fetal Connective Tissue and Cartilage, Collagen, and Silicone: Experimental Study in the Pig with Clinical Results. A New Technique of Dermis Miniautograft Injections," *Aesthetic Plastic Surgery* 14 (1990): 239-48.
85. American Fertility Society, Ethics Committee, "Research on Preembryos: Justifications and Limitations," *Fertility and Sterility* 53 (Suppl. 2)(1990): 62S-63S.

86. B. Gaze and K. Dawson, "Distinguishing Medical Practice and Research: The Special Case of IVF," *Bioethics* 3 (1989): 301-19.
87. E.R. Siebzehnrubl, "Cryopreservation of Gametes and Cleavage Stage Embryos," *Human Reproduction* 4 (Suppl.)(1989): 105-10.
88. Tletze and Henshaw, "Incidence of Abortion."
89. K. Nolan, "Genug Ist Genug: A Fetus Is Not a Kidney," *Hastings Center Report* 18 (December 1988): 13-19.
90. E.D. Thorne and M. Michejda, "Fetal Tissue from Spontaneous Abortions: A New Alternative for Transplantation Research?" *Fetal Therapy* 4 (1989): 37-42.
91. T. Tulandi, "McGill Researchers Have Treated Ectopic Pregnancy Nonsurgically for Two Years," *Medical Post* (5 March 1991), p. 15.
92. A. Drugan et al., "Fetal Organ and Xenograft Transplantation," *American Journal of Obstetrics and Gynecology* 160 (1989): 289-93.
93. Touraine, "Fetal Tissue Transplantation."
94. F.H. Lowy, "Fetal Tissue Transplantation: Time for a Canadian Policy," *Canadian Medical Association Journal* 141 (1989): 1227-29.

Bibliography

American Fertility Society. "Minimal Standards for Programs of In Vitro Fertilization." *Fertility and Sterility* 41 (1984): 13.

American Fertility Society. Ethics Committee. "The Biologic Characteristics of the Preembryo." *Fertility and Sterility* 53 (Suppl. 2)(1990): 31S-33S.

—. "Memory Loss Reversible with Brain Tissue Transplant." *Canadian Doctor* 56 (November 1990): 2.

—. "Research on Preembryos: Justifications and Limitations." *Fertility and Sterility* 53 (Suppl. 2)(1990): 62S-63S.

Auerbach, R. "Qualities of Fetal Cells and Tissues." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health, 1988.

August, C.S., et al. "Implantation of a Foetal Thymus, Restoring Immunological Competence in a Patient with Thymic Aplasia (DiGeorge's Syndrome)." *Lancet* (7 December 1968): 1210-11.

Austin, C.R. "Fertilization." In *Germ Cells and Fertilization*, ed. C.R. Austin and R.V. Short. Cambridge: Cambridge University Press, 1972.

Baird, P.A. "Gene Therapy." *Lancet* (22 April 1989): 902.

Bakay, R.A.E., and D.L. Barrow. "Neural Transplantation for Parkinson's Disease." *Journal of Neurosurgery* 69 (1988): 807-10.

Barrett, D.J., et al. "Clinical and Immunologic Spectrum of the DiGeorge Syndrome." *Journal of Clinical and Laboratory Immunology* 6 (1981): 1-6.

Benoit, B.G., and J.D. Grimes. "Technical Considerations in Brain Grafting for Parkinson's Disease." *Transplantation/Implantation Today* 5 (November 1988): 59-68.

Blakeslee, S. "In Careful Test, Parkinson's Patient Shows Gains After Fetal-Cell Implant." *New York Times* (2 May 1989), sec. C, p. 3.

Bolton, V.N., et al. "Development of Spare Human Preimplantation Embryos In Vitro: An Analysis of the Correlations Among Gross Morphology, Cleavage Rates, and Development to the Blastocyst." *Journal of In Vitro Fertilization and Embryo Transfer* 6 (1989): 30-35.

Brambati, B., and L. Tului. "Preimplantation Genetic Diagnosis: A New Simple Uterine Washing System." *Human Reproduction* 5 (1990): 448-50.

Braude, P., and M. Johnson. "Embryo Research: Yes or No?" *British Medical Journal* (2 December 1989): 1349-51.

Brown, J., et al. "Cryopreservation of Human Fetal Pancreas." *Diabetes* 29 (Suppl. 1)(1980): 70-73.

Brundin, P., et al. "Can Human Fetal Dopamine Neuron Grafts Provide a Therapy for Parkinson's Disease?" *Progress in Brain Research* 78 (1988): 441-48.

—. "Intracerebral Grafting of Dopamine Neurons: Experimental Basis for Clinical Trials in Patients with Parkinson's Disease." *Annals of the New York Academy of Sciences* 495 (1987): 473-96.

Burns, R.S., et al. "A Primate Model of Parkinsonism: Selective Destruction of Dopaminergic Neurons in the Pars Compacta of the Substantia Nigra by N-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine." *Proceedings of the National Academy of Sciences of the United States of America* 80 (1983): 4546-50.

Canadian Fertility and Andrology Society and Society of Obstetricians and Gynaecologists of Canada. *Ethical Considerations of the New Reproductive Technologies*. Toronto: Ribosome Communications, 1990.

Cherfas, J. "Britain's Lords Debate Embryo Research." *Science* 246 (1989): 1554-55.

Cleveland, W.W., et al. "Foetal Thymic Transplant in a Case of DiGeorge's Syndrome." *Lancet* (7 December 1968): 1211-14.

Collier, M., et al. "The Production of Embryo Derived Platelet Activating Factor by Human Embryos and Its Relationship to Pregnancy Outcome." *Clinical Reproduction and Fertility* 5 (1987): 307-308.

Cooper, M.D., R.D.A. Peterson, and R.A. Good. "A New Concept of the Cellular Basis of Immunity." *Journal of Pediatrics* 67 (1965): 907-908.

Cornetta, K., R. Wieder, and W.F. Anderson. "Gene Transfer into Primates and Prospects for Gene Therapy in Humans." *Progress in Nucleic Acid Research and Molecular Biology* 36 (1989): 311-22.

Crombleholme, T.M., et al. "Transplantation of Fetal Cells." *American Journal of Obstetrics and Gynecology* 164 (1991): 218-30.

Drugan, A., et al. "Fetal Organ and Xenograft Transplantation." *American Journal of Obstetrics and Gynecology* 160 (1989): 289-93.

DuPlessis, Y., et al. "A Comparison of Implantation Rates Between Fresh and Frozen-Thawed Embryo Replacement Cycles." In *Proceedings of the Seventh*

Annual Scientific Meeting of the Fertility Society of Australia. Newcastle: Fertility Society of Australia, 1988.

Duvoisin, R.C. *Parkinson's Disease: A Guide for Patient and Family.* 2d ed. New York: Raven Press, 1984.

Fernandez, H., et al. "Spontaneous Resolution of Ectopic Pregnancy." *Obstetrics and Gynecology* 71 (1988): 171-74.

Fine, A. "Transplantation in the Central Nervous System." *Scientific American* 255 (August 1986): 52-58B.

Formigli, L., et al. "Non-Surgical Flushing of the Uterus for Pre-Embryo Recovery: Possible Clinical Applications." *Human Reproduction* 5 (1990): 329-35.

Freed, C.R. "Regarding Transplantation of Fetal Substantia Nigra and Adrenal Medulla to the Caudate Nucleus in Two Patients with Parkinson's Disease." *New England Journal of Medicine* 319 (1988): 370.

Friedmann, T. "Progress Toward Human Gene Therapy." *Science* 244 (1989): 1275-81.

Gale, R.P. "Fetal Liver Transplantation in Aplastic Anemia and Leukemia." *Thymus* 10 (1987): 89-94.

Gale, R.P., and Y. Reisner. "The Role of Bone-Marrow Transplants After Nuclear Accidents." *Lancet* (23 April 1988): 923-26.

Gaze, B., and K. Dawson. "Distinguishing Medical Practice and Research: The Special Case of IVF." *Bioethics* 3 (1989): 301-19.

Goldsobel, A.B., A. Haas, and E.R. Stiehm. "Bone Marrow Transplantation in DiGeorge Syndrome." *Journal of Pediatrics* 111 (July 1987): 40-44.

Gordts, S., et al. "Survival and Pregnancy Outcome After Ultrarapid Freezing of Human Embryos." *Fertility and Sterility* 53 (1990): 469-72.

Gustavii, B. "Fetal Brain Transplantation for Parkinson's Disease: Technique for Obtaining Donor Tissue." *Lancet* (11 March 1989): 565.

Hansen, J.T., and J.R. Sladek. "Fetal Research." *Science* 246 (1989): 775-79.

Harrison, M.R., et al. "In-Utero Transplantation of Fetal Liver Haemopoietic Stem Cells in Monkeys." *Lancet* (16 December 1989): 1425-27.

Hegre, O.D. "Islet Cell Transplantation." In *The Diabetic Pancreas.* 2d ed., ed. B.W. Volk and E.R. Arquilla. New York: Plenum Medical Book, 1985.

Henshaw, S.K., et al. "A Portrait of American Women Who Obtain Abortions." *Family Planning Perspectives* 17 (March/April 1985): 90-96.

Hinderer, U.T., and J. Escalona. "Dermal and Subdermal Tissue Filling with Fetal Connective Tissue and Cartilage, Collagen, and Silicone: Experimental Study in the Pig with Clinical Results. A New Technique of Dermis Miniautograft Injections." *Aesthetic Plastic Surgery* 14 (1990): 239-48.

Hitchcock, E.R., et al. "Embryos and Parkinson's Disease." *Lancet* (4 June 1988): 1274.

Huisjes, H.J. "Spontaneous Abortion." *Current Reviews in Obstetrics and Gynecology*, No. 8. Edinburgh: Churchill Livingstone, 1984.

Hullett, D.A., et al. "Human Fetal Pancreas — A Potential Source for Transplantation." *Transplantation* 43 (1987): 18-22.

Jones, D. "Hospital's Decision to Pursue Fetal Transplantation Upsets Antiabortionists." *Canadian Medical Association Journal* 142 (1990): 1274-77.

Kalter, H. "Diabetes and Spontaneous Abortion: A Historical Review." *American Journal of Obstetrics and Gynecology* 156 (1987): 1243-53.

Kaufman, M.H. "The Origin, Properties and Fate of Trophoblast in the Mouse." In *Biology of Trophoblast*, ed. Y.W. Loke and A. Whyte. New York: Elsevier Science, 1983.

Kochupillai, V., et al. "Fetal Liver Infusion in Acute Myelogenous Leukaemia." *Thymus* 10 (1987): 117-24.

—. "Fetal Liver Infusion in Aplastic Anaemia." *Thymus* 10 (1987): 95-102.

Laitio, M., R. Lev, and D. Orlic. "The Developing Human Fetal Pancreas: An Ultrastructural and Histochemical Study with Special Reference to Exocrine Cells." *Journal of Anatomy* 117 (1974): 619-34.

Le Douarin, N., and A. McLaren, eds. *Chimeras in Developmental Biology*. London: Academic Press, 1984.

Levran, D., et al. "Pregnancy Potential of Human Oocytes — The Effect of Cryopreservation." *New England Journal of Medicine* 323 (1990): 1153-56.

Lindvall, O., et al. "Human Fetal Dopamine Neurons Grafted into the Striatum in Two Patients with Severe Parkinson's Disease: A Detailed Account of Methodology and a 6-Month Follow-Up." *Archives of Neurology* 46 (1989): 615-31.

Lowy, F.H. "Fetal Tissue Transplantation: Time for a Canadian Policy." *Canadian Medical Association Journal* 141 (1989): 1227-29.

McBride, G. "Combo Technology Checks Genes of Preimplanted Embryo." *Medical Post* (5 March 1991), p. 21.

McGourty, C. "Ban on Use of Fetal Tissue to Continue." *Nature* 342 (1989): 105.

McGuire, R. "Parkinson's, Alzheimer's May Share Common Etiology." *Medical Post* (16 October 1990), p. 31.

Madrazo, I., et al. "Transplantation of Fetal Substantia Nigra and Adrenal Medulla to the Caudate Nucleus in Two Patients with Parkinson's Disease." *New England Journal of Medicine* 318 (1988): 51.

Malter, H.E., and J. Cohen. "Blastocyst Formation and Hatching In Vitro Following Zona Drilling of Mouse and Human Embryos." *Gamete Research* 24 (1989): 67-80.

Michael, M., and S. Buckle. "Screening for Genetic Disorders: Therapeutic Abortion and IVF." *Journal of Medical Ethics* 16 (1990): 43-47.

Monk, M. "Embryo Research and Genetic Disease." *New Scientist* 125 (6 January 1990): 56-59.

—. "A Stem-Line Model for Cellular and Chromosomal Differentiation in Early Mouse Development." *Differentiation* 19 (1981): 71-76.

Mullen, M.A., H.W.G. Baker, and W.I.H. Johnston. "A Clinical Trial Utilizing Nycodenz Discontinuous Gradient Preparation for *In Vitro* Fertilisation." *Clinical Reproduction and Fertility* 5 (1987): 289-90.

Mullen, Y.S., et al. "Complete Reversal of Experimental Diabetes Mellitus in Rats by a Single Fetal Pancreas." *Science* 195 (1977): 68-70.

Nolan, K. "Genug ist Genug: A Fetus Is Not a Kidney." *Hastings Center Report* 18 (December 1988): 13-19.

Numazaki, K., et al. "Replication of Measles Virus in Cultured Human Thymic Epithelial Cells." *Journal of Medical Virology* 27 (1989): 52-58.

Ochiya, T., et al. "An *In Vitro* System for Infection with Hepatitis B Virus that Uses Primary Fetal Hepatocytes." *Proceedings of the National Academy of Sciences of the United States of America* 86 (1989): 1875-79.

O'Reilly, R.J., et al. "A Comparative Review of the Results of Transplants of Fully Allogeneic Fetal Liver and HLA-Haplotype Mismatched, T-Cell Depleted Marrow in the Treatment of Severe Combined Immunodeficiency." In *Fetal Liver Transplantation: Proceedings of an International Symposium Held in Pesaro, Italy, September 29 - October 1, 1984*, ed. R.P. Gale, J.L. Touraine, and G. Lucarelli. New York: Liss, 1985.

Pansky, M. "Local Methotrexate Injection: A Non-Surgical Treatment of Ectopic Pregnancy." *American Journal of Obstetrics and Gynecology* 161 (1989): 393-96.

Paulson, R.J., M.V. Sauer, and R.A. Lobo. "Embryo Implantation After Human *In Vitro* Fertilization: Importance of Endometrial Receptivity." *Fertility and Sterility* 53 (1990): 870-74.

Perlow, M.J. "Brain Grafting as a Treatment for Parkinson's Disease." *Neurosurgery* 20 (1987): 335-41.

Phipps, P.H., et al. "Rapid Detection of Influenza Virus Infections in Human Fetal Lung Diploid Cell Cultures." *Journal of Infection* 18 (1989): 269-78.

Quinn, P., et al. "Culture Factors Affecting the Success Rate of *In Vitro* Fertilization and Embryo Transfer." *Annals of the New York Academy of Sciences* 442 (1985): 195-204.

Ratajczak, M.Z. "Experimental Aspects of Transplantation of Haemopoietic Cells of Fetal Liver." *Archivum Immunologiae et Therapiae Experimentalis* 36 (1988): 235-43.

Riordan, J.R., et al. "Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA." *Science* 245 (1989): 1066-73.

Roberts, C.J., and C.R. Lowe. "Where Have All the Conceptions Gone?" *Lancet* (1 March 1975): 498-99.

Sachs, H.H., and M.M. Quigley. "Culture Media for *In Vitro* Fertilization." *Fertility and Sterility* 53 (1990): 953.

Scriver, C.R., et al., eds. *The Metabolic Basis of Inherited Disease*. 6th ed. 2 vols. New York: McGraw-Hill, 1989.

Seibel, M.M. "A New Era in Reproductive Technology: IVF, GIFT, and Donated Embryos and Gametes." *New England Journal of Medicine* 318 (1988): 828-34.

Siebzehnrubl, E.R. "Cryopreservation of Gametes and Cleavage Stage Embryos." *Human Reproduction* 4 (Suppl.) (1989): 105-10.

Sladek, J.R., et al. "Reversal of Parkinsonism by Fetal Nerve Cell Transplants in Primate Brain." *Annals of the New York Academy of Sciences* 495 (1987): 641-57.

Smith, D.H., et al. "Zygote Intra-Fallopian Transfer: The Last Word or the Worst Choice?" *Clinical Reproduction and Fertility* 5 (1987): 400-401.

Soothill, J.F., A.R. Hayward, and C.B.S. Wood, eds. *Paediatric Immunology*. Oxford: Blackwell Scientific, 1983.

Spinks, N.R., et al. "Embryo Derived Platelet Activating Factor: A Mediator for the Establishment of Pregnancy in the Mouse." *Clinical Reproduction and Fertility* 5 (1987): 308-309.

Stegall, M.D., D.E.R. Sutherland, and M.A. Hardy. "Registry Report on Clinical Experience with Islet Transplantation." In *Transplantation of the Endocrine Pancreas in Diabetes Mellitus*, ed. R. Van Schilfgaarde and M.A. Hardy. New York: Elsevier Science, 1988.

Stone, H.B., et al. "Further Reports on Grafting of Endocrine Glands." *Mississippi Doctor* (1938): 6-9.

Sutherland, D.E.R., and K.C. Moudry. "Pancreas Transplant Registry Report — 1986." *Clinical Transplantation* 1 (1987): 3-17.

—. "Pancreas Transplantation Registry Report." *Transplantation Proceedings* 21 (1989): 2759-62.

Thanki, K.H., and C.L. Schmidt. "Follicular Development and Oocyte Maturation After Stimulation with Gonadotropins Versus Leuprolide Acetate/Gonadotropins During In Vitro Fertilization." *Fertility and Sterility* 54 (1990): 656-60.

Thorne, E.D., and M. Michejda. "Fetal Tissue from Spontaneous Abortions: A New Alternative for Transplantation Research?" *Fetal Therapy* 4 (1989): 37-42.

Tietze, C., and S.K. Henshaw. "Incidence of Abortion." In *Induced Abortion: A World Review 1986*. 6th ed. New York: Alan Guttmacher Institute, 1986.

Touraine, J.L., et al. "Fetal Tissue Transplantation, Bone Marrow Transplantation and Prospective Gene Therapy in Severe Immunodeficiencies and Enzyme Deficiencies." *Thymus* 10 (1987): 75-87.

Trounson, A., and L. Freemann. "Role of Cryopreservation of Human Oocytes and Embryos in an IVF Program." In *Progress in Infertility*. 3d ed., ed. S.J. Behrman, R.W. Kistner, and G.W. Patton. Boston: Little, Brown, 1988.

Tuch, B.E., et al. "Recovery of Human Fetal Pancreas After One Year of Implantation in the Diabetic Patient." *Transplantation* 46 (1988): 865-70.

Tulandi, T. "McGill Researchers Have Treated Ectopic Pregnancy Nonsurgically for Two Years." *Medical Post* (5 March 1991), p. 15.

Vines, G. "New Insights into Early Embryos." *New Scientist* 115 (9 July 1987): 22-23.

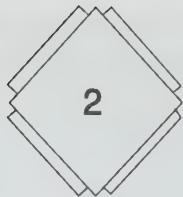
—. "Why Experiment on Human Embryos?" *New Scientist* 124 (4 November 1989): 48-50.

Weber, S. "Fetal Cell Transplant for the Treatment of Parkinson's Disease Scheduled Later in '91." *Medical Post* (15 January 1991), sec. 2, p. 61.

Wellmann, K.F., B.W. Volk, and P. Brancato. "Ultrastructure and Insulin Content of the Endocrine Pancreas in the Human Fetus." *Laboratory Investigation* 25 (August 1971): 97-103.

Wilson, J.D., et al., eds. *Harrison's Principles of Internal Medicine: Companion Handbook*. 12th ed. New York: McGraw-Hill, 1991.

Wright, G., et al. "Observations on the Morphology of Pronuclei and Nucleoli in Human Zygotes and Implications for Cryopreservation." *Human Reproduction* 5 (1990): 109-15.



Legal Issues in Embryo and Fetal Tissue Research and Therapy

Bernard M. Dickens



Executive Summary

A number of legal issues arise from research and therapy involving human pre-embryos, embryos, and fetuses, whether for the benefit of a particular pre-embryo, embryo, or fetus, the benefit of pre-embryos, embryos, or fetuses in general, the benefit of patients through treatment using such tissues, or the development of commercial interests through the use of such tissues.

Research designed to render a particular pre-embryo more successfully implantable *in utero* could raise issues of liability should the pre-embryo survive to be born as a seriously impaired child, including the issue of "wrongful life" suits. It is improbable, however, that criminal liability would arise for gross negligence resulting in the birth of an impaired child, unless that same child could have been born unimpaired.

Research to learn how future pre-embryos, embryos, and fetuses might benefit may deliberately sacrifice the particular pre-embryo, embryo, or fetus on which such research is undertaken. This raises issues of the right of gamete donors to approve research on pre-embryos. Pre-embryo, embryo, and fetal research and therapy could be accommodated or tolerated legally not by designing a specific legislative

framework of permissions and prohibitions, but by creating a regulatory agency to license particular projects and research centres, to approve some types of research or therapy if undertaken in certain classes of facilities, or to approve a particular facility in general to undertake unspecified activities within a given area of competence.

Embryonic or fetal tissues may also be used to treat patients who may or may not be concerned with reproduction. Difficult legal issues arise from the ethical need to ensure that the timing and technique of induced abortion are not influenced by the prospect of using the resulting tissues, lest a woman's wishes or best interests in recourse to abortion be prejudiced or compromised, and that a woman is not influenced unduly to have an abortion rather than to continue a pregnancy. The greatest challenge concerns the scenario — a product more of ethical worst-case speculation than of experience — of a woman who deliberately initiates a pregnancy in order to abort it and donate fetal tissues to an aging parent affected by Parkinson disease or a dependent child in need of organ or tissue transplantation. Legislation is possible if it were concluded that the ethical offensiveness of such an act would warrant legislation to detach abortion decisions from decisions to designate recipients of resulting tissues.

A number of ethical concerns arise to which the law will have to respond if Canadian courts follow a pattern of not recognizing or construing property rights to obstruct the development of commercial health products or services through the use of embryonic or fetal tissues. Unwilling potential consumers of items from embryo and fetal tissue research would have to be protected, for instance through a requirement for labelling or other disclosure devices. Hospital sales of surplus materials unclaimed or released by patients are unobjectionable in principle, although limits may be set against Canadian public hospitals becoming too driven by entrepreneurial enthusiasm to supplement their revenues. More legally questionable may be staff members obtaining personal income from such sales, and private facilities such as abortion clinics trading in the resulting tissues.

Introduction

Medical treatment of human life before live birth can be applied to gametes, preimplantation embryos (pre-embryos), embryos (i.e., following uterine implantation), and fetuses. The Criminal Code recognizes a "child that has not become a human being" (section 238(1)), meaning a fetus of full gestational age, or at least that is independently viable outside the uterus, that is not "in being," meaning that it has not "completely proceeded, in a living state, from the body of its mother" (section 223(1)). This paper considers pre-embryos, both *in vivo* and *in vitro*, and embryos and fetuses *in utero*.

A distinction is commonly drawn in law between "tissues," meaning naturally replaceable body materials such as blood, and "organs," meaning

non-replaceable bodily structures or solid organs such as kidneys and lungs. The distinction is imprecise, because small slivers of bone and areas of skin are naturally replaced *in vivo*, but entire bones and skin surfaces are not; similarly, small liver segments from living donors are replaced *in vivo*, whereas larger liver segments are not. For present purposes, "tissues" will include "organs," and distinctions between replaceable tissues and non-replaceable organs will be specified where necessary.

Legal issues concerning research and therapy involving human pre-embryos, embryos, and fetuses arise from a number of different activities, including

- research and therapeutic innovation intended to enhance or reduce the prospect of implantation and gestation of a particular pre-embryo;
- research and therapeutic innovation intended to enhance the gestation, viability, and healthy birth of a particular embryo or fetus;
- research intended to improve or reduce the prospects of implantation and gestation of pre-embryos in general, such as studies of implantation failure;
- research intended to improve the prospects of gestation, viability, and healthy birth of embryos and fetuses in general, such as studies of spontaneous abortion and defects in newborn children;
- research and therapeutic innovation using embryonic or fetal tissues intended to benefit born patients, such as neonates, persons with genetic or congenital anomalies, and patients affected by neurological diseases such as Parkinson and Alzheimer disease;
- therapeutic procedures using embryonic or fetal tissues intended to benefit patients, such as transplantation of fetal bone marrow or organs; and
- research intended to use embryonic or fetal tissues to develop medically and otherwise employable commercial products, such as cell lines, or to develop biotechnological services.

This paper addresses four individual categories:

1. treatment of particular pre-embryos, embryos, and fetuses;
2. treatment to benefit pre-embryos, embryos, and fetuses in general;
3. treatment to benefit patients through the use of embryonic or fetal tissues; and
4. the development of commercial interests through the use of embryonic or fetal tissues.

The following overview of legal issues within these categories will not be supported by case law or legislative references, except where specifically necessary. Further, being directed to the law, the paper does not generally discuss whether procedures that are legally permissible are ethically objectionable or prohibited.

Treatment of Particular Pre-Embryos, Embryos, and Fetuses

The Pre-Embryo *In Vitro*

Research designed to render a particular pre-embryo successfully implantable *in utero* raises no direct legal problem when it is undertaken by informed and free agreement of the gamete contributors and, if she is not the ovum source, of the woman in whom implantation is proposed. Implantation in such a woman raises issues of what has come to be called surrogate motherhood (whether undertaken on an altruistic, commercial, mixed, or other basis), but not issues about consensual implantation. An ovum source must give equally informed and free consent, of course, to be implanted with a treated pre-embryo.

Indirect legal issues concern liability if the research renders a particular pre-embryo implantable and capable of full-term gestation and live birth as a human being, but not capable of experiencing a life free of gross handicaps. If a pre-embryo survives to be born as a seriously impaired child, its legal claim might be a conventional claim that, but for negligence, it would have been implanted, gestated, and born to a normal state; or, it might be an unconventional claim that it suffered wrong because it should never have been implanted, gestated, or born alive. The former claim would be difficult to establish on a balance of probabilities, because of the relatively high implantation failure rate of even normal pre-embryos. The latter claim is often described as a "wrongful life" claim, and a general presumption in Canadian law, reflecting pre-1980 United States experience, has been that a claim of this sort would not be accommodated. It has recently been held, however, that such a claim is allowable.¹

Liability for "wrongful life" could restrict a practice that might develop to obviate the need for abortions. A couple might decide, rather than risk pregnancy with a fetus afflicted with, say, Tay-Sachs disease, and abort it if prenatal diagnosis disclosed the disorder, to seek preimplantation diagnosis. They would achieve *in vitro* fertilization (IVF) and have a cell removed from each eight-cell pre-embryo for genetic diagnosis. Unaffected pre-embryos could be implanted with the prospect of birth of one or more unaffected children, and affected pre-embryos would be discarded. If negligent preimplantation diagnosis resulted in implantation of an affected pre-embryo and the eventual birth of an affected child, however, his or her legal action for wrongful life might succeed. In several of the U.S. states,

legislation claiming to uphold the sanctity of human life prohibits courts from allowing wrongful life claims. The appropriateness of such provincial or territorial legislation in Canada may warrant consideration.

It is highly improbable that criminal liability would arise for gross negligence so resulting in the birth of an impaired child. The offence, under section 221 of the Criminal Code, of criminal negligence causing bodily harm would fail if any negligence caused the birth of the child rather than its liability to suffer injury or harm. Similarly, no liability for the death of such a child might arise under section 223(2), which provides that "[a] person commits homicide when he causes injury to a child before or during its birth as a result of which the child dies after becoming a human being." Negligence in facilitating birth would not be the cause of the injury, and in any event it is improbable that a pre-embryo would be considered in law to be "a child before ... its birth."

Non-consensual implantation in a woman to preserve the viability of a particular pre-embryo would be a violation of criminal laws and civil laws on aggravated assault (perhaps including sexual assault) and battery. It is doubtful that the doctrine of necessity to save human life could excuse such an assault or battery in law, since a court is highly unlikely to consider that the objective weight given to preserving a particular pre-embryo outweighs the preservation of a woman's physical integrity against the violation of attempted implantation.

More worthy of attention is non-consensual treatment of a pre-embryo to enhance its prospect of successful implantation in a consenting woman other than the source of the ovum. The necessity argument may weigh more favourably against a gamete donor's claim of a property interest to compel the discard of a treatable pre-embryo. In the absence of clear law on the status of a pre-embryo *in vitro*, property law principles may be applicable; laws equating the pre-embryo with a born child and invoking, for instance, "best interests" concepts regarding custody and protection against child abuse quickly lead to absurdity, as U.S. legislative and case-law experience has shown.² Contractual arrangements between physicians and gamete sources should be required or encouraged, and in principle their terms should prevail. Doctrine on necessity may, however, excuse violations of both property rights and contractual undertakings.

A medical or biological scientist may be legally excused for treating a pre-embryo without consent if it was done to offer the ovum donor a reasonable prospect of successful implantation and normal gestation and birth of that particular pre-embryo. It is questionable whether such treatment could be undertaken in defiance of an express refusal of consent by the ovum source, given that the source would be apt to decline to receive implantation. It is less likely that the refusal of the sperm source would warrant the same legal regard when the ovum source approved treatment of the pre-embryo and/or its implantation in her. Even if she expressly refused both implantation in her and initial treatment of the pre-embryo, another issue warrants attention: should its non-consensual preparation

for implantation in an alternative, willing recipient be legally accommodated? The analogy may be clumsy, but owners of historically or architecturally valuable buildings are often restrained legislatively from destroying them, notwithstanding their ownership. Owners of pre-embryos may appropriately suffer similar legal restraints on destruction or wastage. Weighing heavily in the balance, however, are gamete sources' legal protections against involuntary parenthood and imposition on them of the legal and moral duties parents owe children.

The Pre-Embryo, Embryo, and Fetus *In Vivo*

Research, therapeutic innovation, and therapy intended to enhance the normal gestation and birth of a particular pre-embryo, embryo, or fetus within a woman's body raise legal issues that have come to be considered under the rubric of maternal-fetal conflict. It should first be observed, however, that no conflict arises when a woman gives informed and free consent to treatments intended for this purpose. How free a woman is in fact to decline the offer of treatment and to risk spontaneous abortion, stillbirth, or the birth of a severely impaired child, or to choose induced abortion, is a matter of fact that legal processes can be designed to monitor. Standards of disclosure and procedures to reinforce informed and free consent can be laid down by legislation if desirable.

The issues of civil liability for "wrongful life" and of criminal liability addressed earlier in this paper are also applicable here, but with an important variant concerning fetuses of advanced gestational age. It is more credibly argued in the case of a child born alive that it was injured because of negligent, though well-intentioned treatment administered late in pregnancy, without which it would have been born alive without such injuries. Similarly, criminal liability may arise for criminal negligence causing bodily harm that a child born alive might not otherwise have suffered, if the injury is associated with gross or reckless negligence in treatment administered late in pregnancy, and if the fetus might be viable or of full gestational age so as to be "a child before ... its birth" within the meaning of Criminal Code section 223(2) on homicide. No liability for criminal negligence causing death would arise if stillbirth occurred, nor for causing bodily harm to a fetus that failed to survive birth.³

It is now unlikely that Canadian courts would apply child protection legislation to *in vivo* products of conception so as to compel women to undertake protective conduct, such as refraining from certain types of employment. However, third parties, such as prospective employers whose work environments might be teratogenic (or harmful to a fetus), might be subject to occupational health and safety legislation designed to protect embryos and fetuses *in vivo*. Exclusion of pregnant women from the workplace would raise issues of sexual discrimination.

Women fearful of embryonic or fetal loss or injury, and who are willing to submit to protectively intended medical treatments, are free to do so,

subject as ever to their informed and free consent. Fetal surgery conducted *in utero*, or by removing the fetus from the uterus for treatment and replacing it, is now feasible, and in itself faces no legal obstacles. A legal question concerns the status of a fetus removed from the uterus for treatment with a view to replacement. The Criminal Code expresses the general common law in providing that a child becomes a human being when it has proceeded in a living state from the body of its mother, whether or not it has breathed, has an independent circulation, or the navel string is severed (section 223(1)). This condition is satisfied when a fetus is wholly removed for surgery before replacement. It must therefore be asked whether it has become a human being and whether its later induced abortion is thus criminally chargeable as murder, or its accidentally caused injury or loss is chargeable as criminal negligence causing bodily harm or manslaughter.

Legislation could be considered to maintain the historical position by providing that status as a human being follows from spontaneous or induced permanent birth, but not induced removal from the uterus with the intention of replacement. That leaves the question of the status of a viable fetus, removed with a view to being replaced *in utero*, when replacement is not undertaken but the fetus is placed instead in an incubator as a prematurely born child.

Treatment to Benefit Pre-Embryos, Embryos, and Fetuses

Research to learn how future pre-embryos, embryos, and fetuses might benefit may deliberately sacrifice the particular pre-embryo, embryo, or fetus on which such research is undertaken.

Planned wastage of pre-embryos raises no legal question when undertaken with gamete donors' consent. The legal authority with which gamete donors may approve wastage of surplus or other unimplanted pre-embryos created in IVF programs confirms their right to approve research on pre-embryos. They may also approve research aimed at promoting implantation. This raises the question of whether they may first permit research on a pre-embryo and then undertake experimental implantation, intending from the outset to terminate any resulting pregnancy. The historical law of maim (or mayhem) prevents individuals from consenting to non-therapeutic bodily mutilation, but it is not obvious that this branch of criminal prohibition applies to experimental pregnancy. Accordingly, initiation of voluntary pregnancy for purposes of research designed to terminate pregnancy and inspect the products of conception appears not to violate the law.

It must be asked whether legal limits should govern such activity. The guidelines of the Medical Research Council of Canada⁴ contemplate

research on a pre-embryo or embryo of up to 17 days' gestation and perhaps more. No criminal law currently limits abortion, but provincial laws and regulations governing the practice of medicine apply both to the unqualified practice of medicine and to unethical practice by licensed physicians. Thus, those who terminate pregnancies on grounds that offend the public interest in the ethical practice of medicine may lawfully be called to account.

No Canadian legislation directly prohibits implantation in a human of an animal or mixed human-animal hybrid pre-embryo. Provincial regulation of medical practice may be invoked, however, to prevent or sanction such an occurrence; to promote public values, the practice might be brought within the criminal law, as it has been in other jurisdictions. Similarly, placing human or hybrid pre-embryos in animals might be criminalized, if it is not already subject to the law against wilfully injuring cattle or other animals. Against this, the Law Reform Commission of Canada has urged that criminal laws not be made in order to govern improbable or speculative conduct.

Destructive research on pre-embryos without the express consent of the gamete sources raises legal questions about whether such sources abandoned the pre-embryos to the lawful control of the experimenters or retained a possessory interest in them, so implicating criminal law on theft of or causing mischief to property and related civil (provincial) laws. Agreements between gamete sources and IVF clinics may afford patients sufficient control over unimplanted pre-embryos and disposal of unimplantable or surplus pre-embryos to determine the legality of destructive research. Nevertheless, federal criminal law or provincial law on health care or property could be considered, either to operate in default of an agreement or to supersede any objectionable agreement. Such legislation might require, for instance, that any research intention be communicated explicitly, and consent be given explicitly, and perhaps recorded, before destructive research is initiated. Legislation could also address payments to gamete sources, relief from sources' costs of clinical services, and payments of charges and incidental expenses of third parties who facilitate acquisition of pre-embryos for research.

The prevailing body of law relevant to human medical research, addressing, for instance, issues of consent, governs destructive or potentially destructive or teratogenic research on an embryo or fetus *in utero*. The same law governs experimental research on a woman, including therapeutic innovation designed for her benefit that may incidentally endanger or compromise an embryo or fetus *in utero*. Complaints have recently arisen that medications have not been tested on women who are, who may be, or who may become pregnant, for fear of the testing causing embryonic or fetal damage or loss. As a result, pregnant women for whom these medications are prescribed or available over the counter cannot be informed about their safety, correct dosages, or risks. Researchers have therefore resumed recruitment of pregnant women in research trials that

pose little risk; but no trial can ever be free from all risk. This may raise questions of whether the embryos or fetuses thereby become research subjects, and whether their live but impaired births are legally actionable through wrongful injury or wrongful life actions, or criminally punishable.

Canadian law permits women to terminate pregnancies in accordance with provincial medical licensing authorities' codes of ethical medical practice. Women may, within these limits, plan pregnancies and their terminations in compliance with research protocols. More problematic is whether women who receive implantation of non-therapeutically treated pre-embryos may continue gestation and give birth without legal liability. Civil liability might be for wrongful birth; criminal liability might be for criminal negligence causing bodily harm or, if the child dies following birth, causing death. There is no legal liability to a fetus as such, since a child must be born alive in order to become in law a human being, but it acquires status upon live birth however premature and non-viable it may be. The law now regards living products of human conception to be human beings, having discarded the medieval category of monsters. These were grossly disfigured or unrecognizably human products of conception, which in the Christian tradition received only conditional baptism ("If thou art human, I baptize thee").

In infertility treatments, for instance for failure of implantation or chronic spontaneous abortion, it may be difficult to categorize treatment as research, therapeutic innovation, diagnosis, or therapy, and to determine whether it is for the benefit of a couple or either of them, for that of a particular pre-embryo, embryo, or fetus, or that of a prospective product of conception whether *in vitro*, *in vivo*, or *in utero*. A study of what triggers spontaneous abortion or implantation failure, for instance, may be aimed at its specific prevention or at its close monitoring in order to prevent it at a future occasion.

A danger of drawing up different rules for different practices is that practices may be obstructed that do not fit clearly within the categories drawn. Experience in the United States shows, moreover, that research practices prohibited under federal regulations concerning pre-embryos and embryos can be undertaken under the description of clinical diagnosis of reproductive failure. Similarly, legal prohibitions on creating pre-embryos for the sole purpose of research are inapplicable to research into infertility that seeks to initiate conception of a couple's gametes and pre-embryonic implantation. The prohibition may be on creating a pre-embryo predestined for destruction in the cause of research, including research that may facilitate future successful pregnancies.

Pre-embryo, embryo, and fetal research and therapy could be accommodated or tolerated legally not by designing a specific legislative framework of permissions and prohibitions, but by creating a regulatory agency to license particular projects and research centres, to approve some types of research or therapy if undertaken in certain classes of facilities, and, for instance, to approve a particular facility in general to undertake

unspecified activities within a given area of competence. The model of the Human Fertilisation and Embryology Authority, set up in the United Kingdom under the Human Fertilisation and Embryology Act 1990, clearly warrants attention, perhaps in the context of comparable and contrasting authorities elsewhere, such as in Victoria, Australia. A review of like options would have to consider the relevance and possible impact of the *Canadian Charter of Rights and Freedoms*.

Treatment to Benefit Patients Through the Use of Embryonic or Fetal Tissues

The type of research just addressed, employing pre-embryos, embryos, or fetuses, can be considered diagnostic or therapeutic treatment of a couple's or a patient's infertility, the product of conception being only a means to this end. In this third category of research, diagnosis or therapy is part of a wider range of medical treatments in which embryonic or fetal tissues are used to treat patients who may or may not be concerned with reproduction. Fetal tissues sometimes include placentas produced in the delivery of healthy children, but more often the phrase refers to such materials as solid organs recovered from more advanced fetuses for transplant into sick children, and fetal neural cells for use in patients with Parkinson disease.

Many legal issues concerning the therapeutic and research use of such tissues are novel in their application but not in themselves. Legal control or ownership of such tissues, whether they become available by spontaneous or induced termination of pregnancy, is resolved through traditional principles of criminal and particularly civil property law, contract law, the law of fiduciary relationships, and the law of constructive and resulting trusts. The commercial surrender, acquisition, and transfer of these tissues raise issues of whether contracts could be declared invalid if they contradicted public policy or violated laws governing gifts of human tissue. More difficult legal issues arise from the ethical need to ensure that the timing and technique of induced abortion are not influenced by the prospect of using the resulting tissues, lest a woman's wishes or best interests in recourse to abortion be prejudiced or compromised, and that the woman is not influenced unduly to have an abortion rather than to continue a pregnancy. Also applicable here are doctrines on free and informed consent to whether, when, or how to have an abortion, on undue influence or inducement, and on physicians' and others' obligations to disclose adverse or conflicting interests.

Women must also be legally free to decide on ethically appropriate donations of embryonic and fetal tissues that may assist others. For instance, a woman who determines to continue a pregnancy when the fetus has been shown by ultrasound or otherwise to be anencephalic, so that on

birth and rapid natural death its organs can be harvested and transplanted, must be afforded legal means to do so. More challenging is the case of a woman who wishes to schedule an abortion to maximize the prospects of fetal tissues becoming available at the right time to assist others. The greatest challenge concerns the scenario — a product more of ethical worst-case speculation than of experience — of a woman who deliberately initiates a pregnancy in order to abort it and donate fetal tissues to another, such as an aging parent affected by Parkinson disease or a dependent child in need of organ or tissue implantation. This is often described as the problem of the "designer fetus."

The ethics of prohibiting and accommodating specific designation of transplantable tissues will fashion the law in this area. Currently, no laws prevent women from initiating pregnancies how, when, and why they want to, subject to laws against incest, which bar sexual intercourse, but not artificial insemination, within prohibited degrees of family proximity. Similarly, laws permit women to terminate pregnancies how, when, and why they want to, subject to legal monitoring of medical and other health practitioners' professional ethics. A woman who can commence a pregnancy to serve the perceived needs of an existing single child, or to please a parent's wish for grandparenthood, and who can end a pregnancy to serve the needs of her own health, can both start and end a pregnancy to meet the perceived benefit and health of others.

If it is concluded, however, that the ethical offensiveness of allowing designer fetuses warrants legislation to detach abortion decisions from decisions to designate recipients of resulting transplantable fetal tissues, such legislation can be proposed. The prevention of tissue designation can be approached directly, by criminal or non-criminal sanctions against prospective donors, or indirectly, by administrative regulation of health professionals or facilities.

The same means are available, and can be reinforced for instance through an appropriate legally authorized institutional or personal licensing system, to restrain other research or therapeutic initiatives considered to be ethically objectionable. The idea, for instance, of undertaking harmful research on a fetus in anticipation of its abortion may be objectionable not simply in itself but also because it compromises a woman's choice to forgo abortion and continue a pregnancy. Techniques can be developed by which to give legal effect to values and preferences that are drawn from ethical perceptions.

The likelihood of fetal tissues becoming available for transplantation, including both neural cells and solid organs, will be reduced considerably if antiprogestin drugs, such as the French abortion drug RU-486, become widely available and used in Canada. This is already a widely used technique of choice in medical abortion in France; it can be anticipated that the same will occur in Canada once the drug is licensed. Abortions using this drug occur earlier in pregnancy than would accommodate tissue use and usually at home, so that tissue recovery is compromised. Asking a

woman to forgo this non-surgical abortion technique in favour of a more invasive, less convenient, and less acceptable method so as to produce usable tissues raises legal issues of inviting women to suffer inconvenience and related costs for the benefit of unknown others; these issues are comparable to those raised in medical research. Ethically, it might be unlikely that this could be approved as research, which raises the question of whether any prohibition of coercive or inducive pressure should be left to ethical control or be implemented or reinforced by legislation. Similarly, the role of monetary inducements of consent to forgo a preferable treatment warrants legal attention to both prevailing and prospective relationships between health professionals, patients, and facilitating and service personnel.

Development of Commercial Interests Through the Use of Embryonic or Fetal Tissues

The legal accommodation and regulation of commercial biotechnology are already influencing courts and challenging legislatures. The much-discussed 1990 decision of the California Supreme Court in *Moore v. Regents of the University of California*⁵ was founded explicitly on the interest of not obstructing potentially commercially rewarding biotechnological innovation by imposing obligations on investigators to ensure that sources of human tissues had given their informed and free consent to their availability for culturing and other development. The Court held that conventional doctrines of attending physicians' duties to make adequate disclosure of any interest in conflict with their patients' wishes and best interests were available and adequate to resolve any claims of patients who became engaged in medical procedures designed to benefit their physicians' research and commercial interests rather than their own interests in health, bodily integrity, and autonomy. Patients' claims to property interests in their commercially (as opposed to therapeutically) recovered tissues were rejected on the basis of the Court's instrumental reasoning that favoured the liberation of biotechnological research.

The Court supported its decision by reference to a California law on the duty to dispose of pathological waste, but its general reasoning and orientation fit into a common law setting and are relevant to Canada. If Canadian courts follow a pattern of not recognizing or construing property rights to obstruct the development of commercial health products or services through the use of embryonic or fetal tissues, a number of ethical concerns will arise to which the law will have to respond. One is the objection by those who feel that use of such tissues encourages or rewards abortion, and recourse to products or services from them amounts to complicity in abortion. A protection of unwilling potential consumers of items produced from embryo and fetal tissue research would be the legal

requirement that such products and, where possible, therapeutic services be identified as such, through labelling or other disclosure devices. Then, in the same way that Jehovah's Witnesses avoid use of blood-derived products, consumers or patients could avoid the use of products derived from embryonic and fetal tissue. This is subject to the application of child protection laws requiring parental consent for life- or health-preserving treatments of dependent children or permitting displacement of parents as medical decision makers for their children.

Another issue concerns hospital or clinic sales of placentas and other fetal or embryonic tissues to commercial and industrial organizations. Hospital sales of surplus materials unclaimed or released by patients are unobjectionable in principle, although limits may be set against Canadian public hospitals becoming too driven by entrepreneurial enthusiasm to supplement their revenues. Income from by-products of their practices that subsidizes health services delivered by hospitals is lawfully acquired. More legally questionable may be staff members obtaining personal income from such sales, and private facilities such as abortion clinics trading in the resulting tissues. Some evidence from the United States indicates that private abortion clinics may reduce charges to low-income clients in return for clients' release of any claims to fetal or other removed tissues. Such arrangements, which are not inconceivable in Canada, would raise legal questions about patients' control of the timing and method of the abortions offered, about counselling practices, and about patients' capacities to change their minds.

Neither patients, health professionals, nor health facilities can sell tissues as such, because of provisions of provincial law governing gifts of human tissues. Distinguishable from commodity sales, however, are service transactions. A patient who obtains abortion services may appear to sell resulting tissues if she receives payment, but a medical recipient of her fetal and related tissues who lawfully recovers them, preserves them, differentiates them, diagnoses them, prepares them for transportation, and transports them may recover fees for such services. Thus, a woman cannot lawfully be paid, in cash or in kind, for delivering her fetal tissues, but a lawful recipient, by her abandonment or gratuitous release of such tissues, may charge for the service of making them available to others. A profit motive may appear absent when a remunerated health facility is legally established on a not-for-profit basis. It may be observed, however, that such facilities may lawfully be established by health professionals who hold staff positions for which they receive generous fees, fees that are reflected in charges for facility services. Thus, although the facilities themselves are not run for commercial gain, they are sources of considerable personal enrichment to proprietors who are also staff members. Regulatory or licensing legislation may open such practices to public scrutiny and control.

Abbreviations

B.C.S.C.	British Columbia Supreme Court
Cal.Sup.Ct.	California Supreme Court
C.C.C.	Canadian Criminal Cases
D.L.R. (4th)	Dominion Law Reports (Fourth Series)
P.2d	Pacific Reporter (Second Series)
S.C.C.	Supreme Court of Canada

Notes

1. *Cherry (Guardian) v. Borsman* (1991), 75 D.L.R. (4th) 668 (B.C.S.C.), appeal pending.
2. See B.M. Dickens, "Comparative Judicial Embryology: Judges' Approaches to Unborn Human Life," *Canadian Journal of Family Law* 9 (1990): 180-92.
3. *R. v. Sullivan* (1991), 63 C.C.C. (3d) 97 (S.C.C.).
4. Medical Research Council of Canada, *Guidelines on Research Involving Human Subjects* (Ottawa: Minister of Supply and Services Canada, 1987).
5. *Moore v. Regents of the University of California* (1990), 793 P.2d 479 (Cal.Sup.Ct.).

Bibliography

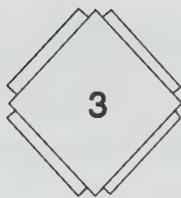
Cherry (Guardian) v. Borsman (1991), 75 D.L.R. (4th) 668 (B.C.S.C.), appeal pending.

Dickens, B.M. "Comparative Judicial Embryology: Judges' Approaches to Unborn Human Life." *Canadian Journal of Family Law* 9 (1990): 180-92.

Medical Research Council of Canada. *Guidelines on Research Involving Human Subjects*. Ottawa: Minister of Supply and Services Canada, 1987.

Moore v. Regents of the University of California (1990), 793 P.2d 479 (Cal.Sup.Ct.).

R. v. Sullivan (1991), 63 C.C.C. (3d) 97 (S.C.C.).



Human Fetal Tissue Research: Origins, State of the Art, Future Applications, and Implications

Alan Fine



Executive Summary

Tissue from human fetal cadavers has long been used for medical research, experimental therapies, and other purposes. Research within the last two decades has led to substantial progress in many of these areas, particularly in the application of fetal tissue transplantation to the treatment of human disease. As a result, clinical trials have now been initiated at centres around the world to evaluate the use of human fetal tissue transplantation for the therapy of Parkinson's disease, insulin-dependent diabetes mellitus, and a number of blood, immunologic, and metabolic disorders.

Laboratory studies suggest a much wider range of disorders may in the future be treatable by transplantation of various types of human fetal tissue. A combination of characteristics renders fetal tissue uniquely valuable for such transplantation, as well as for basic research, the development of vaccines, and a range of other applications. Although substitutes for human fetal cadaver tissue are being actively sought, no satisfactory alternatives are currently available for many of these applications.

Important unresolved issues remain concerning the procurement, distribution, and use of human fetal cadaver tissue as well as the effects of such use on abortion procedures and incidence. These issues can be addressed by the introduction of appropriate guidelines or legislation, and need not be an impediment to legitimate research and therapeutic use of fetal tissue.

Introduction

Human fetal tissue has been used for research and experimental therapeutic purposes throughout this century. Advances in different fields of biology and medicine over the last two decades have resulted in a growing list of present and potential uses of fetal tissue. At the same time, vigorous public debate on abortion has focussed attention on government policy — or the lack of policy — regarding the use of fetal tissue.

In this report, past and present research using human fetal cadaver tissue — tissue that may be living but that is removed from a dead fetus or embryo — is reviewed, with emphasis on tissue transplantation and other areas in which clinical applications are clearly envisaged or already under way. Potential future uses of, and alternatives to, human fetal cadaver tissue are also outlined. Ethical and social implications of the use of such tissue are then considered, and recommendations are made for government policy in this area. Research on the intact living fetus or embryo, or on the placenta or fetal membranes, is outside the scope of this report.

Relevant Properties of Fetal Tissue

Although it is self-evident that human fetal tissue is the appropriate material for studies of aspects of human fetal biology, fetal cells and tissues possess other characteristics that render them uniquely useful for other purposes such as transplantation. Several relevant general characteristics can be identified (American Medical Association 1990; Auerbach 1988).

Fetal cells and tissues have the ability to undergo change and to differentiate, in response to environmental cues or according to their own intrinsic program. This "plasticity" can include growth, elongation, migration, and establishment of functional connections with other cells. Such plasticity is progressively restricted during the course of normal development, and is largely lost in many tissues by the end of fetal development. Thus, for example, the extent of brain innervated by transplanted nerve cells has been shown to decrease sharply with increasing age (from fetal to post-natal) of the donor (Seiger and Olson 1977).

Cells in many fetal tissues, depending on gestational stage, are still proliferating. In general, a larger fraction of cells are capable of division in fetal than in more mature tissues, and they can divide more rapidly and more often.

Fetal cells may produce high levels of various substances — including angiogenic factors that induce blood vessel formation and neurotrophic factors necessary for neuronal survival — that can enhance their growth as grafts and that may also facilitate regeneration by surrounding host tissues (Björklund et al. 1987).

Fetal tissue may elicit a weaker immune response than the corresponding fully mature tissue. In some cases, this may be due to the late expression of major antigens during development (Auerbach 1988; Geyer et al. 1985); however, this is not a uniform characteristic of fetal tissue (Garvey et al. 1979; Simeonovic et al. 1980; Tuch 1988). In other cases, this may reflect the absence, or easy elimination, from the fetal tissue of highly antigenic passenger cells such as leucocytes, vascular endothelia, or dendritic cells that are present in the fully differentiated tissue. An immunologic consideration of special importance for haematopoietic transplantation, discussed below, is the immaturity of the early fetal immune system; grafts of early fetal haematopoietic tissue lack mature lymphocytes, and thus do not recognize and attack tissues of the "foreign" recipient (graft-versus-host reaction).

Many fetal cells are able to survive at lower oxygen levels than their fully differentiated counterparts, which renders them more resistant to ischaemic damage both during *in vitro* manipulations and after transplantation. Proliferating or immature fetal cells generally lack long extensions or strong intercellular adhesions; thus, they are also less subject to traumatic injury during excision, dissection, and dissociation into suspensions of individual cells. Such characteristics simplify handling of fetal tissue, permitting tissue to be transplanted, for example, by injection of a cell suspension instead of by surgical implantation of intact tissue. Also, these characteristics may account for the enhanced survival of fetal cells and tissues, compared to those of the adult, after refrigeration or frozen storage (cryopreservation) (Wong 1988).

A healthy uterine environment protects fetal tissue from exposure to pathogens; as a result, suction abortion can produce tissue that, although disrupted, is ordinarily viable and uncontaminated (Markowski and Lawler 1977). Moreover, abortion is a common procedure in many countries including Canada, so that human fetal cadaver tissue is often significantly more abundant than tissue from adult cadavers.

Research and Therapeutic Uses of Human Fetal Tissue: Review and State of the Art

It will be useful for the purposes of this paper to consider transplantation applications of fetal tissue, in which the fetal tissue itself is used for the direct benefit of a patient, separately from all other uses, including various forms of research as well as development and testing of biologic or pharmaceutical products.

Uses of Human Fetal Tissue Other Than Transplantation

The use of human fetal tissue has been essential for studies concerned explicitly with aspects of human fetal development and differentiation (Peel et al. 1972; Hansen and Sladek 1989). Routine examination of fetal cadaver tissue for evidence of developmental abnormalities may represent the largest research use of fetal tissue; however, because results are often kept, unpublished, in institutional registries, relevant statistics are unavailable (Vawter et al. 1990). These and other anatomical studies generally have been performed on dead or preserved material. Other studies, generally involving *in vitro* culture of living tissue or cells, have been carried out to elucidate biochemical and physiologic processes in normal human development (Colten 1972; Tuddenham et al. 1974; Rowley et al. 1978; Zimmermann et al. 1979; Rayfield et al. 1987; Clark and Kamen 1987). Human fetal cells have also long been standard preparations for the propagation, study, and diagnosis of certain human pathologic viruses and other micro-organisms, and for the study of mechanisms in cancer induction, vascular degeneration, and other disease processes (Peel et al. 1972). Such *in vitro* application of human fetal tissue has provided important information on aspects of viral biology and the pathogenesis of human diseases (Dolin et al. 1970; Mitus et al. 1970; Phipps et al. 1989; Ochiya et al. 1989), and has been instrumental in the production of vaccines against polio, rubella, and other diseases (American Medical Association 1990; Haase 1987).

Human fetal tissue has been used to create "models" of human disease for subsequent research. Human fetal haematopoietic stem cells can survive transplantation to immunodeficient mice. Although these transplanted animals have played a role in developing transplantation therapies for human immune disorders (see below), they have also yielded important information about human haematolymphoid differentiation and function (McCune et al. 1988; Namikawa et al. 1990; Vandekerckhove et al. 1991; Krowka et al. 1991), and have led to the creation of models of human leukemias (Dick 1991) and acquired immunodeficiency syndrome (AIDS) (Namikawa et al. 1988; McCune et al. 1990). The ability to construct human-animal heterochimeras by transplantation of fetal tissue is not restricted to the haematopoietic system; human fetal intestine has been

transplanted to immunodeficient mice to study the development of the gut (Winter et al. 1991).

The Medical Research Council of Canada (MRC) does not maintain as a distinct category specific records of its support of research involving human fetal tissue; thus, it is not possible to specify the precise extent of this support. However, interviews with Canadian scientists indicate that the MRC has provided funds totalling several million dollars over the last three decades for research in Canada using human fetal cadaver tissue. This MRC-supported research has used tissue from over 1 000 fetuses for the investigation of a wide range of subjects, such as the regulation and effects of fetal hormone secretion, the normal and pathologic development of fetal organs, and aspects of fetal tissue metabolism including generation or elimination of toxic or therapeutic compounds. Non-governmental sources have supported similar research in other Canadian laboratories. In total, more than 25 research groups in Canada have used human fetal tissue for their investigations during this period. At least 10 of these groups have worked with tissue of the fetal nervous system, studying aspects of neural outgrowth, remyelination, trophic factor interactions, gene expression, transplantation, enzyme activity, ontogeny and physiology of hormone secretion, human immunodeficiency virus (HIV) infection, and neuroimmunologic interactions. Fetal adrenal tissue has been used in at least 10 laboratories for studies of development and regulation of fetal steroid production, gene expression, transplantation, enzyme activity, and binding and effects of other hormones. At least seven groups have used fetal liver tissue to study aspects of haematopoiesis, effects of hormones on liver development, enzyme activities, expression of growth factor-related genes, and metabolism of drugs used in the treatment of premature babies. At least seven have used fetal lung tissue to study lung development, regulation of surfactant production, trophic factors, hormone and drug effects, gene expression, enzyme activity, and effects of maternal smoking on lung development. At least six groups have used fetal kidney tissue for studies of hormonal effects on kidney development, and of gene expression and enzyme activities in the developing kidney. At least five groups have used tissues from the human fetal gastrointestinal tract to study hormonal and other influences on gut development and cell proliferation, the regulation of fetal bile production, development of intestinal nutrient absorption, and patterns of gene expression. At least four laboratories have used fetal thymus, muscle, and spleen for studies of gene expression and the pathophysiology of HIV infection. At least three groups have used fetal heart tissue to study developmental changes in gene expression and enzyme activity, and to investigate the role of endothelin in human cardiac development. Fetal skin has been used in at least two laboratories for studies of gene expression and hormonal influences in development. Fetal pancreas has been used in at least one laboratory, for the *in vitro* culture of islets of Langerhans, to study the development of glucose responsiveness. One laboratory has used fetal cartilage tissue for the study of proteoglycans

of human joints in development and disease. (In addition, at least 15 laboratories have used placenta or fetal membranes for various purposes; this paper, dealing with tissue from human fetal cadavers, will not further consider uses of placenta or fetal membranes, although their use may raise certain issues in common with that of fetal tissue.) Many of these studies are currently in progress (Medical Research Council of Canada 1991). As far as can be determined, all research in Canada using human fetal tissue has been done at universities, hospitals, or other non-profit institutions, using tissue collected from local hospitals with ethical review board approval.

In Great Britain, where fetal human tissue has been supplied to approved researchers since 1957 by the Medical Research Council Tissue Bank, records are available describing the type and frequency of different research uses (Lawler 1981; Wong 1988). From 1981 to 1986, fetal cadaver tissues, including adrenal gland, bone marrow, brain, heart, kidney, liver, lung, intestines, nasal mucosa, ovary, pancreas, peripheral nerve, salivary gland, skeletal muscle, skin, spleen, testis, thymus, and trachea, were distributed to 124 users. Of these, 29 percent were involved in virologic and bacteriologic investigations, including studies of tissue susceptibility and effect of vaccines; 20 percent in immunologic and haematologic investigations, including studies of lymphocyte development, antigen expression, and haematopoiesis; 23 percent in molecular biologic and genetic investigations, including studies of tissue-specific, developmentally regulated patterns of gene expression; 15 percent in tissue culture and physiologic investigations, including studies of development of excitability and of cell-cell interactions; and the remainder in other studies, including descriptive embryology and anatomy. In each of these years, approximately 700 disrupted specimens were used, derived from pregnancies terminated by suction; approximately 100 specimens derived from prostaglandin-induced abortions were also used each year, although the tissues from these specimens are generally less viable (Markowski and Lawler 1977).

It has been reported that fetal cells are used by biotechnology, pharmaceutical, and other companies to screen new pharmaceutic agents for toxicity or to identify teratogens or carcinogens (Hansen and Sladek 1989; Vawter et al. 1990), but these claims are difficult to substantiate, reflecting a lesser degree of public accountability that may distinguish corporate from institutional use of fetal tissue. Fetal meninges are reported to be given to pharmaceutical companies in Holland (Gunning 1990), although sources for this report are not given. Allegations that human fetal cadaver tissue has been used for the production of cosmetics ("Embryos" 1985) were found to be groundless, or reflect confusion over the use of placenta or fetal membranes collected after birth for various purposes, including preparation of skin care products. A cosmetic industry representative assured the Legal Affairs Committee of the Council of Europe Parliamentary Assembly in 1986 that "neither the French cosmetic industry nor the European, American or Japanese industries have ever envisaged

using fetuses for their manufactures" (Haase 1987). Investigators have used human fetal cadaver tissue at academic institutions for *in vitro* testing of the potential mutagenic, carcinogenic, or teratogenic effects of various compounds or their fetal metabolites (Lasnitzki 1968; Juchau and Namkung 1974; Juchau et al. 1972, 1978; Berry et al. 1977; Pynnonen et al. 1977; Namkung et al. 1977; Jones et al. 1977; Chin et al. 1979; Pacifici and Rane 1982; Rettie et al. 1986); however, the U.S. National Library of Medicine data base (MEDLARS 1970 to present) contains no reports of such research using human fetal tissue conducted at industrial laboratories. A pharmaceutical industry representative informed the Council of Europe that the industry did not use embryos or their tissues for therapeutic purposes (Haase 1987), and in a survey of some 70 Canadian biotechnology and drug companies conducted for this paper through the auspices of the Pharmaceutical Manufacturers Association of Canada, none of the 34 responding companies indicated any past, present, or planned use of human fetal tissue for any purpose, nor any interest in possible uses, including drug development and safety testing, in Canada. It is not known whether such use is under way or planned in non-responding companies. Furthermore, responses from international companies were restricted, in some cases explicitly, to Canadian operations.

Indeed, notwithstanding these responses, reliable sources within the pharmaceutical industry and agencies distributing fetal tissue have confirmed that there is industrial interest in the use of human fetal cadaver-derived tissue for certain purposes, and that such tissue has been received by industrial laboratories in the United States, including those of, or affiliated with, companies whose Canadian subsidiaries responded to our survey. The largest distributor of fetal tissue for research in the United States, the International Institute for the Advancement of Medicine (IIAM), has supplied this material to 86 institutions: 67 were non-profit, and 19 were for-profit corporations, whose researchers were engaged in studies of toxicology, metabolism, gene expression, the development of diagnostic tools, or (in three instances) therapeutic techniques (presumably transplantation) (B.M. Bardsley, IIAM executive director for research services, pers. comm.). An informal survey in 1988 by the National Abortion Federation, with responses from over half of its 300 U.S. clinic members, revealed that 11 clinics provided fetal cadaver tissue for research programs, which in two cases were carried out by for-profit commercial laboratories (Vawter et al. 1990). Two for-profit biotechnology companies currently receive human fetal cadaver tissue from the University of Washington's Laboratory of Human Embryology (LHE), a second major distributor of fetal tissue for research (A. Fantel, LHE director, pers. comm.). The director of Advanced Bioscience Resources, Inc. (ABR), another major fetal tissue distributor, has indicated that while academic laboratories represent approximately 75 percent of the groups receiving human fetal cadaver tissue from that agency, more tissue is used in total

by the smaller number of industrial laboratory recipients (L. Tracy, ABR director, pers. comm.).

In his book on global traffic in commodities, Ridgeway (1980) reported that human fetal cadaver tissue has been shipped from South Korea to Fort Detrick, Maryland, for use by the U.S. Army in investigations of haemorrhagic fever, with potential application to biologic warfare, and that "as many as 100 000 fetuses a year end up in research laboratories" around the world. Although this report has been cited as evidence of "a rapidly growing market for human fetuses" (Roberts 1988), this author has been unable to establish its basis or to verify its accuracy. Similarly, Scott, in *The Sunday Times* of London, reported in 1977 that "between 1970 and 1976 a South Korean medical practitioner had sold 12 000 pairs of fetal kidneys to an American medical supply corporation at an average price of \$15 a pair ... A spokesman for the corporation which bought them said that it acquired fetal tissues from some 250 sources in 12 countries, and that the kidneys, which were in great demand, were resold in the United States to various laboratories and hospitals doing research aimed at producing antivirus vaccines" (Scott 1981).

Human Fetal Tissue Transplantation: Research and Clinical Implementations

Transplantation of human fetal cadaver tissue has generated more public interest and controversy than any other use. This public attention has kept pace with the increasing rate of scientific progress on fetal tissue transplantation over the last two decades. However, fetal tissue transplantation research, aimed at a wide range of disorders, has been carried out throughout this century. The following review summarizes past and current use of human fetal tissue for transplantation, with reference to animal experiments where relevant.

Endocrine (Other Than Neuroendocrine) Disorders

Attempts to correct endocrine disorders by transplantation were among the earliest uses of human fetal tissue. Endocrine disorders have seemed particularly suitable for transplantation therapy since living endocrine tissue cells could be expected to regulate hormone release in response to appropriate cues, thereby maintaining more effective homeostasis than can be achieved through simple hormone replacement therapy using orally administered or injectable hormones.

Adrenal

Transplantation of adrenal glands appears to have been first attempted in animals as early as 1887 (Canalis 1887), and fetal adrenal allografts (i.e., grafts between genetically non-identical members of the same species) were performed in rabbits in 1904 (Parodi 1904). Clinical applications of human fetal adrenal transplantation for the treatment of adrenal cortical insufficiency (Addison's disease) were reported in 1922 (Hurst et al. 1922)

and 1935 (Bailey and Keele 1935), with continuing benefit to the patients in both cases. However, subsequent studies (Woodruff 1953, 1960) have indicated that clinical improvement after fetal adrenal transplantation is not correlated with graft survival. Patients with Addison's disease are well maintained by oral corticosteroids, and there appears to be little current interest in North America or Europe in transplantation of human fetal adrenal tissue for this purpose. However, in China, 13 patients with Addison's disease and unsatisfactory response to prednisone were successfully treated between 1985 and 1990 by transplantation of adrenal glands from spontaneously aborted fetuses of six to eight months' gestational age (Yan et al. 1990). All patients were maintained on immunosuppressive medication; after 3 to 21 months, all were described as recovered or showing significant improvement, with no evidence of graft rejection.

Transplantation of fetal adrenal medullary tissue, for therapy of Parkinson's disease, will be considered later.

Thyroid

The history of thyroid transplantation has parallels with that of adrenal tissue. Although transplantation of (non-fetal) thyroid tissue was widely used earlier in the century as a treatment for various forms of thyroid deficiency (Woodruff 1960), indications for such transplants have been eliminated by the more satisfactory results of oral thyroid hormone administration. Nonetheless, interest in clinical application of thyroid transplantation continues in some countries, and research on the preparation of human fetal thyroid tissue for transplantation purposes continues (Blyumkin et al. 1988).

Pancreas

The use of fetal pancreatic tissue for the treatment of diabetes mellitus has been suggested by numerous investigators, including Banting and Best (Bliss 1982). The failure of standard insulin replacement therapy to meet the changing homeostatic demands of the patient can lead to significant and life-shortening complications, including kidney disease, cardiovascular disease, and blindness, which might be avoided by the transplantation of endocrine pancreas (islets of Langerhans). Fetal pancreas has long been considered to be an attractive source of tissue for transplantation because of its relative enrichment in endocrine tissue (or precursors) with respect to exocrine tissue (as compared to the adult organ), its potential abundance, and its potentially lesser immunogenicity. In addition, transplantation of fetal pancreas before a critical period of exocrine development leads to degeneration of exocrine cells and generation of pure endocrine tissue (Brown et al. 1984), reducing the need for difficult islet purification procedures required for transplantation of adult tissue.

Human fetal pancreas was first transplanted in 1928 (Fichera 1928); the patient, an 18-year-old man with diabetes mellitus, did not improve, and died in a diabetic coma three days after the operation. Efforts to treat

insulin-dependent (type I) diabetes mellitus by fetal pancreas transplantation continued intermittently without success (Stone et al. 1938; Brooks and Gifford 1959). Interest in this approach was rekindled by the first unambiguous demonstration of reversal of experimental diabetes by fetal pancreas grafts in rodents by Brown et al. (1974) and Mullen et al. (1977). Subsequently, human fetal islet tissue has been found able to survive, develop, and restore normal blood glucose levels in immunodeficient rodents rendered experimentally diabetic (Usadel et al. 1980; Shumakov et al. 1980; Tuch et al. 1984a, 1984b, 1988; Tuch and Monk 1991; Hullett et al. 1987; Bethke et al. 1988; Elias et al. 1990; Tuch and Osgerby 1990; Tuch 1991). To date, the principal reported application of human fetal pancreas has been in laboratory studies aimed at optimizing the preparation of such tissue for future transplantation (Vawter et al. 1990; Hellerstrom et al. 1989; Lissing et al. 1988; Hullett et al. 1989; Leonard et al. 1989).

On the basis of these observations, human fetal pancreas allografts to patients with insulin-dependent diabetes mellitus have been attempted around the world, beginning in 1977 (Groth et al. 1980; Usadel et al. 1980). By 1991, over 1 500 insulin-dependent diabetic patients had received transplants of fetal pancreas tissue into various sites (Hellerstrom et al. 1988; Peterson et al. 1989; Vawter et al. 1990; Federlin et al. 1991). Most were in the former Soviet Union and the People's Republic of China (Benikova et al. 1987; Suskova et al. 1988; Rozental et al. 1988); unfortunately, many of the reports from these countries lack details necessary for critical evaluation. About 16 percent of the recipients showed a measurable increase in serum levels of C peptide, an indicator of insulin secretion; fewer than 2 percent no longer needed exogenous insulin, in some cases as much as 45 months after transplantation (Federlin et al. 1991).

Endocrine pancreas cells are detectable in the human fetus as early as the eighth week of gestation (Stefan et al. 1983; Reddy and Elliott 1988) and are organized into islets as early as the fourteenth week (Hahn von Dorsche et al. 1984, 1990); the relative and absolute numbers of insulin-secreting cells increase thereafter. Studies *in vivo* and *in vitro* indicate that this development is sensitive to various growth factors, including insulin itself (Sandler et al. 1985, 1987a, 1987b, 1989; Formby et al. 1988; Tuch and Lenord 1989; Peterson et al. 1989; Eckhoff et al. 1991), the absence of which may have contributed to the relatively poor results of fetal pancreas transplants to date. Because of the small mass of insulin-secreting cells in the fetal pancreas, most clinical transplants have used pooled tissue from as many as 24 fetuses of 16 to 20 weeks' gestational age per recipient (Vawter et al. 1990). To accumulate these numbers, cryopreservation and storage at -196° C (Mazur et al. 1976; Kemp et al. 1978; Shiogama et al. 1990; Kuhn et al. 1990) have been used in some cases (Dawidson et al. 1988).

Inadequate immunosuppression also may have contributed to the poor success rate of early fetal pancreas transplants. The immunogenicity of fetal pancreatic tissue may be due mainly to the presence of "passenger" cells, including cells of the immune system (Danilovs et al. 1982; Hahn von Dorsche and Falt 1990), vascular endothelium, and duct epithelium (Motojima 1989). Efforts have been directed toward eliminating these cells from endocrine pancreatic tissue before transplantation; for example, by enzymatic disruption of donor vasculature during islet isolation and by interposing a period of tissue culture before grafting (Simeonovic et al. 1980; Mandel 1984; Ricordi et al. 1987; Cochrum et al. 1989; Simpson et al. 1991). It has also been determined that major histocompatibility antigens important in allograft rejection (class II: human leucocyte antigen [HLA]-DR) are less abundant in human fetal islets younger than 18 weeks' gestational age (Motojima et al. 1989). The ability to HLA-type human fetal tissue beyond 14 weeks' gestational age (Danilovs et al. 1983; Tuch et al. 1985) may permit reduced rejection by transplantation of human fetal islets to matched recipients. Clinical transplants using cultured islet cells, more potent immunosuppression, or both, have produced claims of long-lasting reduction in patients' insulin requirement (Rozental et al. 1988). Encapsulation within semi-permeable membranes could provide another means of protecting islets from rejection, without recourse to immunosuppression, while permitting free passage of glucose and insulin (Lim and Sun 1980). Encapsulated human fetal islets have been shown to survive and function after xenotransplantation, maintaining normoglycaemia for extended periods in diabetic rodents (Wu et al. 1989). In a preliminary clinical trial, encapsulated human fetal islets were allografted by intraperitoneal injection to three insulin-dependent diabetic patients; patients' insulin requirements were reduced and evidence of graft-dependent insulin secretion was obtained post-operatively, but these changes persisted less than six months (*ibid.*).

Haematopoietic Disorders, Inherited Metabolic Diseases, and Other Diseases Treated by Transplantation of Fetal Liver or Thymus

A wide range of life-threatening disorders result from the absence or dysfunction of particular classes of blood cells. Cure may be achieved by transplantation of healthy stem (precursor) cells able to generate a perpetual supply of the appropriate peripheral blood cells. The usual source of such stem cells is bone marrow; transplantation of histocompatible bone marrow is now the treatment of choice for many of these disorders. Unfortunately, the limited supply of appropriately histocompatible HLA-matched donors makes such life-saving transplants unavailable for most potential recipients. Rejection in the context of bone marrow is remarkable in that not only can the graft be rejected by the host, but the graft can also cause a catastrophic graft-versus-host (GVH) disease. This abnormality occurs when immunocompetent histoincompatible graft cells attack tissue of an immunodeficient host, resulting in multisystem failure.

Indeed, in the presence of pathologic or therapeutic immunosuppression, GVH disease, which may be fatal, is frequently the major barrier to successful transplantation.

In the course of fetal development, precursors to the blood-forming stem cells arise in the primitive yolk sac at about the fourth week of gestation. They migrate to the fetal liver by the sixth week of gestation, moving thereafter to the thymus, spleen, and bone marrow (Metcalf and Moore 1971). Thus, between 4 and 18 weeks' gestation, the fetal liver is a concentrated source of pluripotent haematopoietic stem cells (Gale 1987a, 1987b). The particular advantage of fetal liver as a source of haematopoietic stem cells for transplantation is the immunologic immaturity of this tissue. Lymphocytes capable of eliciting GVH disease are found in the developing human liver only after the eighteenth week of gestation (O'Reilly et al. 1983). During maturation, lymphocytic precursors recognizing "self" antigens are eliminated; thus, stem cells derived from sufficiently early-gestation fetal liver might correct blood disorders without causing GVH disease. Such grafts would still be subject to rejection by the host if the host immune system is functional. For this reason, fetal liver transplantation has been attempted mainly in patients with non-functional immune systems: for treatment of immunodeficiency disorders, as replacement therapy after bone marrow depletion due to administration of anti-neoplastic drugs or exposure to radiation, and for diseases that can be diagnosed *in utero* (including inborn errors of metabolism) when the fetal recipient's immune system is also immature.

By 1958, the ability of fetal liver haematopoietic stem cells to reconstitute the depleted immune system had been demonstrated in rodents, rescuing them from otherwise lethal whole body irradiation without inducing GVH disease (Uphoff 1958); in subsequent animal studies, transplantation of fetal liver resulted in permanent cure of a hereditary haematologic disease, macrocytic anaemia (Seller and Polani 1966). Before this, in 1957, human bone marrow from a 26-week-gestational-age fetus was injected intravenously in an unsuccessful attempt at haematopoietic reconstitution after total-body irradiation therapy for chronic myeloid leukemia (Thomas et al. 1957). Unsuccessful transplantation of fetal human liver cells after total-body irradiation for disseminated seminoma was described by Woodruff in 1960; temporary engraftment of fetal human haematopoietic stem cells was reported in the same year by Bridge et al. In 1961, two of four patients with chronic pancytopenia treated by injection of fetal human haematopoietic cells were reported in remission, although evidence of successful engraftment was lacking (Bodley et al. 1961).

In 1968, two children with a rare thymic aplasia (DiGeorge's syndrome) were successfully treated by transplantation of human fetal thymus tissue (August et al. 1968; Cleveland et al. 1968). Fetal human liver and thymus were transplanted to infants with combined immunodeficiency disease by several groups (Harboe et al. 1966; Githens et al. 1973), and the

first successful immunoreconstitution with human fetal liver cells, of an infant with severe combined immunodeficiency disease (SCID) due to adenosine deaminase deficiency, was achieved by Keightley et al. in 1975.

Since then, human fetal liver has been used in laboratory and animal studies aimed at improving the viability, function, and storage of haematopoietic stem cells for transplantation (Simonova et al. 1976; Groscurth et al. 1986; Afanasyev et al. 1989; Phan et al. 1989; Lavrik et al. 1990). Fetal thymus transplantation is now the therapy of choice for complete DiGeorge's syndrome (Goldsobel et al. 1987; Touraine et al. 1987), and has been used successfully in conjunction with transfer factor for the treatment of thymic hypoplasia with abnormal immunoglobulin synthesis (Nezelof syndrome) (Ammann et al. 1975) as well as chronic mucocutaneous candidiasis due to immunodeficiency (Ballow and Hyman 1977). Fetal liver transplantation (with or without fetal thymus) has, in the absence of HLA-matched bone marrow donors, been used for treatment of haematopoietic disorders, including SCID (Buckley et al. 1976; O'Reilly et al. 1980; Loudon and Thompson 1988; Touraine et al. 1991), aplastic anaemia (Kansal et al. 1979; Kelemen 1973; Harousseau et al. 1980; Kochupillai et al. 1987a; Han et al. 1990), and acute myelogenous and lymphoblastic leukemias (Lucarelli et al. 1980; Izzi et al. 1985; Kochupillai et al. 1987b). By 1987, at least 122 transplants of fetal cadaver livers had been performed for aplastic anaemia and 39 for acute leukemias (Gale 1987a, 1987b). Improvements were reported in 54 percent of the aplastic anaemia patients, but engraftment could be confirmed in only 3 percent of the cases; recovery after fetal haematopoietic transplantation without evidence of successful engraftment suggests the possibility that lymphokines or other chemical immune-stimulating factors present in the grafts, rather than surviving cells, may have been responsible for the patients' improvement. In contrast, at least transient engraftment was demonstrable in 41 percent of the leukemia patients; in these patients, immunosuppression due to high-dose chemotherapy, irradiation, and the disease process presumably was responsible for the lower rate of graft rejection than in the aplastic anaemia patients with intact immune systems. Human fetal liver has also been transplanted, without beneficial effect, to severely irradiated victims of the Chernobyl reactor disaster (Gale and Reisner 1988; Baranov et al. 1989).

These disorders may be treated more successfully by transplantation of T lymphocyte-depleted bone marrow from partially matched related donors (Vossen 1988; O'Reilly et al. 1988), but this may be so only where more predictable availability of related-donor bone marrow allows better preparation of recipients (O'Reilly et al. 1988). No fatal cases of GVH disease have occurred in patients receiving transplants of human fetal liver haematopoietic stem cells of less than 14 weeks' gestational age (Crombleholme et al. 1990; Golbus and Bauer 1990), and the success rate for these transplants may be increasing. In a recent summary of a large series, Touraine et al. (1991) reported the results of transplanting human fetal haematopoietic tissue, chiefly fetal liver of 8 to 12 weeks' gestational

age, to 58 infants with severe immunodeficiency diseases, severe aplastic anaemia, or unspecified inherited metabolic disease. Of those with immunodeficiency diseases, 50 percent were reported to be completely cured or significantly improved after 1 to 16 years. Despite mismatch of donor and recipient major (class I and II) histocompatibility antigens, immune response to pathogenic organisms by these patients several years after transplantation was normal (Touraine 1983).

Immunodeficiency diseases may result from genetic disorders: SCID, for example, can result from a defective gene for adenosine deaminase. With the development of improved molecular biologic tools, it is now possible to diagnose many genetic disorders *in utero*, at a time when immaturity of the host immune system may facilitate successful engraftment. Correction of a mouse genetic haematologic disease (macrocytic anaemia) *in utero* was first described in 1979 by Fleischman and Mintz, and two human fetal patients with inherited severe immunodeficiency disorders (Bare Lymphocyte Syndrome and SCID) have been treated subsequently by infusion of human fetal liver and thymus cells (Touraine et al. 1989) into the umbilical vein *in utero*; successful engraftment was reported in the absence of GVH disease (Touraine 1989). More recently, the same technique has been used to treat a non-immune haematopoietic disorder, thalassemia (Touraine et al. 1991).

In these genetic haematologic disorders, the purpose of fetal liver transplantation is to supply a source of normal blood cells. In other genetic disorders, fetal liver cells have been proposed as sources of normal proteins. In so-called "storage" disorders, absence of functional enzymes leads to build-up of unmetabolized substrates in various cells, with pathologic consequences; fetal liver cells transplanted to these patients may secrete the missing enzyme, which could be taken up by the patients' cells, correcting their defective metabolism. Fetal haematopoietic tissue transplantation has been reported for treatment of 28 patients with inborn errors of metabolism, including Gaucher's disease, Fabry's disease, fucosidosis, Hurler syndrome (alpha-L-iduronidase deficiency), metachromatic leukodystrophy, Hunter's syndrome, glycogenosis, Sanfilippo's B syndrome, Morquio B syndrome, and Niemann-Pick disease types A, B, and C, with an overall survival rate of 77 percent 1 to 16 years after transplantation (Touraine 1989; Touraine et al. 1987, 1991). Treatment of Hurler syndrome by *in utero* transplantation of fetal liver cells has also been attempted by Slotnick and colleagues (Peck 1991; Culliton 1992), although it is too early for the outcome to be determined.

Human fetal liver cells have been transplanted similarly to correct an acquired metabolic disorder, hepatic insufficiency due to hepatitis B infection (Zhu et al. 1990); improvements in patients' liver function were reported, but evidence of engraftment was not described.

Neurologic Disorders

Transplantation of mammalian brain tissue was first attempted before 1890 by Thompson; survival of immature brain tissue allografts was reported by Dunn in 1917. Attempts at brain tissue transplantation continued over the next decades. Faldino (1924) reported survival of fetal mammalian central nervous system tissue transplanted to the anterior eye chamber, and Le Gros Clark (1940) reported survival of the tissue transplanted to the brain. Neurons and glia of the fetal brain lack major (class I and II) histocompatibility antigens under normal circumstances (Wong et al. 1984), although these antigens are expressed on brain endothelia (Mauerhoff et al. 1988) and meningeal cells, and may be induced in certain glia by gamma-interferon and other stimuli (Wong et al. 1984, 1985; Mauerhoff et al. 1988). Thus, in contrast to other fetal tissue (Hofman et al. 1984), grafts of properly prepared fetal neural cells may survive transplantation between unrelated individuals of the same species without rejection.

Serious efforts to reconstruct the damaged or diseased brain by fetal neural tissue transplantation began only after the development of new anatomical marking techniques that permitted the unambiguous demonstration of graft survival (Olson and Seiger 1972; Das and Altman 1971) and, especially, after the demonstration that grafts could influence host brain function in an animal model of a neurodegenerative disease (Perlow et al. 1979; Björklund and Stenevi 1979). At this time, clinical application of fetal neural transplantation has been mainly restricted to Parkinson's disease.

Parkinson's Disease

Parkinson's disease is a devastating neurologic disorder that results from degeneration of neurons in the midbrain substantia nigra. Release of dopamine from fibres of these cells into the forebrain striatum — essential for normal initiation and execution of movement — is impaired as a result of this degeneration. The causes of the degeneration are usually unknown, and there is no cure. Although some other brain pathways are also affected, most, including the targets of the dopamine fibres, are relatively spared, making this disorder particularly amenable to reconstruction by fetal neural transplantation; substantial behavioural improvements have been obtained when rat fetal dopaminergic brain tissue was implanted into the dopamine-depleted striatum of rats with an experimentally induced form of parkinsonism (Perlow et al. 1979; Björklund and Stenevi 1979; Björklund et al. 1981). These results have been widely confirmed and extended in rodents (Nadaud et al. 1984) and primates (Sladek et al. 1987; Fine et al. 1988; Dunnett and Annett 1991). In addition, human fetal dopaminergic brain tissue has been found to reduce motor deficits upon intrastriatal transplantation in immunosuppressed parkinsonian rats (Stromberg et al. 1986; Brundin et al. 1986). The mechanisms by which intracerebral fetal neural grafts exert these behavioural effects are not fully

understood, but evidence shows that they can supply missing neurotransmitter or neuromodulator substances not only by diffuse release but by reformation of anatomically appropriate synaptic connections with neurons in the host brain. In addition, the grafts may produce growth-stimulating factors, stimulate such production by the host brain, influence gene expression and other aspects of metabolism of host brain neurons, and serve as physical conduits for regeneration of host brain pathways (Fine 1986; Björklund et al. 1987; Segovia et al. 1989, 1991).

In 1987, before the publication of primate results, clinical trials of human fetal dopaminergic brain tissue transplants were begun in Sweden (Lindvall et al. 1988, 1989), Mexico (Madrazo et al. 1988), and England (Hitchcock et al. 1988). Encouraging results and no major complications have led to the continuation of these trials (Lindvall 1991a, 1991b; Lindvall et al. 1990, 1992; Sawle et al. 1992; Madrazo et al. 1990a, 1990b, 1991a; Hitchcock et al. 1990a, 1990b, 1991; Clough et al. 1990) and to the initiation of similar clinical trials in Cuba (Molina et al. 1991), Spain (Lopez-Lozano et al. 1991), the United States (Freed et al. 1990, 1991; Redmond et al. 1990; Vawter et al. 1990), Canada (A. Fine, unpublished observations), France (M. Peschanski, principal investigator, pers. comm.), China (Blakeslee 1989), Czechoslovakia (Subrt et al. 1990), and Poland (Dymecki et al. 1990). These preliminary clinical trials, and their potential implications for wider application of fetal tissue transplantation to the treatment of other disorders, have been major causes of the current upsurge in public attention and controversy concerning the use of human fetal cadaver tissue. As of August 1992, more than 120 severely afflicted parkinsonian patients have been treated by intrastriatal implantation of fetal ventral mesencephalic brain tissue, usually of 6 to 12 weeks' gestational age, using material from 1 to 16 fetuses per patient. (In general, transplanted fetal neurons survive best when taken from embryos shortly after their last cell division, but before outgrowth of extensive axons and dendrites that would be traumatized during dissection; for human fetal dopaminergic neurons of the substantia nigra, this period appears to be mainly between five and eight weeks' gestational age [A. Fine, unpublished observations; Freeman and Kordower 1991; Brundin and Sauer 1991].) Improvements have been reported in most cases, and in no case have symptoms worsened (Madrazo et al. 1991c; Thompson 1992a). However, inadequate documentation and lack of standardization have made it difficult to evaluate most of these claims, and even well-documented reports have been criticized (Freed 1990; Miletich et al. 1990).

Human fetal sympathetic neurons and adrenal chromaffin cells, which under certain circumstances may synthesize and secrete dopamine, have been transplanted to the striatum of parkinsonian monkeys, but have had no effect on their clinical symptoms (Yong et al. 1989). Fetal adrenal tissue has also been transplanted to the striatum of three parkinsonian patients (Madrazo et al. 1990a, 1990b, 1991a); however, results have been disappointing (Marsden 1991), and this procedure has been discontinued.

Huntington's Disease

Huntington's disease is a progressive, autosomal-dominant inherited disease, characterized by dyskinesias and mental deterioration resulting from massive degeneration of striatal neurons, particularly the inhibitory projection neurons that use gamma-aminobutyric acid as a transmitter substance. Although symptoms of the disease generally appear only in the third or fourth decade, newly available genetic markers permit identification of carrier or afflicted people even *in utero*. There is no effective treatment, and death generally occurs by age 50. Results of studies in rodents (Deckel et al. 1983; Isaacson et al. 1984, 1986) and primates (Hantraye et al. 1990) have demonstrated that fetal striatal grafts to the lesioned striatum can survive and provide partial functional restitution to the lesioned animal. As in Parkinson's disease, clinical trial of human fetal neural transplantation has been initiated on the basis of rodent studies before publication of primate results (Madrazo et al. 1991b); slight motor improvements in the first patient have been reported after one year.

Multiple Sclerosis

Multiple sclerosis, a chronic disease of unknown etiology, is the most common of a number of disorders in which myelin surrounding central nervous system axons degenerates. This myelin is produced by oligodendrocytes; in contrast to the myelin-producing Schwann cells of the peripheral nervous system, these cells are incapable of proliferation. No cure is yet available for multiple sclerosis, and though most cases are characterized by episodic relapse and remission, others deteriorate progressively. Transplants of rodent fetal brain tissue (Gumpel et al. 1983) or purified fetal oligodendrocytes (Rosenbluth et al. 1990) in myelin-deficient rats and mice have induced myelin formation around axons of the host brain and spinal cord. Human fetal oligodendrocytes have also been found capable of producing myelin after transplantation to myelin-deficient mice (Gumpel et al. 1987), raising the possibility of remyelinating affected regions of central nervous systems of multiple sclerosis patients by focal transplantation of human fetal oligodendrocytes. Functional effects of such fetal glial transplants have not been evaluated; clinical trials have not been attempted, and it is unclear if transplanted oligodendrocytes would be subject to the disease process during episodes of relapse.

Schizophrenia

Recently, it has been claimed in a disturbing report that fetal brain tissue transplants have been used for the treatment of schizophrenia (Kolarik and Nadvornik 1989, cited in Rafael and Ayulo 1991). Details of the operation and its outcome are not available; there is no justification for such a procedure on the basis of current understanding of schizophrenia.

Cosmetic and Reconstructive Surgery

Because of their capacity for growth, fetal skin, connective tissue, and bone have been considered for use in plastic or reconstructive surgery. Successful construction of a neovagina using abdominal skin, vagina, and uterine tissue from spontaneously aborted fetuses of five to five and one-half months' gestational age has been described in two cases of vaginal aplasia with normal ovaries (Rokitansky-Küstner-Hauser syndrome) (Luisi 1978). Anatomical and functional integrity of the grafts was noted up to seven years after transplantation; remarkably, although grafts were apparently not HLA-matched to the recipients nor were the recipients immunosuppressed, no evidence of rejection was found (*ibid.*; Fanciulli 1978). Notwithstanding the good results claimed in these reports, this procedure appears not to have been used subsequently.

Demineralized metaphyseal bone from human fetuses of 11 to 16 weeks' gestational age have been transplanted to athymic rats to assess their potential as an inductive matrix to stimulate new bone formation (Aspenberg and Andolf 1989); their inductive capacity was not greater than that of adult bone matrix. More promising results have been obtained using viable mouse fetal epiphysis for hemiepiphyseal reconstruction in newborn mice (Wolohan and Zaleske 1991).

Future Applications of Human Fetal Tissue

Transplantation

Laboratory investigations over the last decade demonstrate interest in developing fetal tissue transplantation therapies for a wide range of disorders beyond those described previously. Progress in investigations of these diverse applications is varied, as is the likelihood of their clinical realization.

Neurologic, Ophthalmologic, and Muscular Disorders

Because nerve cells of the adult brain and spinal cord cannot proliferate, and because their capacity to regenerate damaged fibre pathways is limited, recovery from central nervous system trauma or disease is often poor. No effective treatments are available for many neurologic disorders; some are progressive and fatal. In the view of many neurologists and neuroscientists, fetal neural transplantation is a promising approach to the restoration of function in certain of these disorders (Fine 1986; U.S. Congress 1990; Lindvall 1991a, 1991b).

The results of animal experiments suggest that, in addition to the movement disorders of Parkinson's and Huntington's diseases, the dementias of Alzheimer's disease, advanced Parkinson's disease, and chronic alcoholism (Korsakoff's syndrome) may be responsive to appropriate fetal neural transplantation. At present, these dementias are untreatable.

The pathology of Alzheimer's disease is conspicuously widespread, involving many cortical and subcortical brain regions; however, profound degeneration of certain monoamine-secreting pathways, particularly of the acetylcholine-secreting projections from the basal forebrain to the neocortex and hippocampus, is characteristic of all three of these disorders (Davies and Maloney 1976; Mann and Yates 1983; Arendt et al. 1983), and may be correlated with the extent of cognitive deficits (Perry et al. 1985; Collerton 1986). Experimental studies in rats (Flicker et al. 1983; Murray and Fibiger 1985) and monkeys (Ridley et al. 1986) have provided further evidence that degeneration of the acetylcholine-secreting projections may contribute to memory impairment in these disorders. So far, it has not been possible to reproduce the full pathology of these human diseases in experimental animals. Nevertheless, memory impairments due to chronic ethanol administration (Arendt et al. 1983) or disruption of acetylcholine projections to neocortex (Fine et al. 1985) or hippocampus (Dunnett et al. 1982) in rats and monkeys (Ridley et al. 1991) have been restored by transplantation of fetal neurons of the acetylcholine-producing basal forebrain to the depleted cortex or hippocampus; fetal neural transplant-mediated restoration of another monoamine, serotonin, to the depleted rat hippocampus has also ameliorated memory impairments (Nilsson et al. 1990; Richter-Levin and Segal 1989).

Preliminary experimental studies in rodents suggest that at least two other types of neurodegenerative disorders, motor neuron diseases such as amyotrophic lateral sclerosis and hereditary degenerative ataxias, may be amenable to treatment by fetal neural transplantation (U.S. Congress 1990; Lindvall 1991a, 1991b). These progressive, untreatable, and fatal disorders are characterized by degeneration of spinal motoneurons and cerebellar neurons, respectively. In studies using animal models of these diseases, it has been demonstrated that fetal spinal motoneurons transplanted into the experimentally motoneuron-depleted spinal cord of adult rats can establish anatomical interactions with the host (Nothias et al. 1990; Sieradzan and Vrbova 1991), while fetal cerebellar Purkinje's cells transplanted into the cerebellum of Purkinje's cell-degeneration mutant mice can establish features of normal cerebellar circuitry (Sotelo and Alvarado-Mallart 1991). An ability of these grafts to induce functional recovery has not yet been demonstrated.

Although most forms of epilepsy are controllable by medication, at least 10 percent of patients will not respond adequately; surgical removal or isolation of epileptic foci can control seizures in some of these patients, although resulting impairments may be profound. Results of animal experiments suggest that fetal neural transplantation may provide an alternative and less destructive means for control of some types of medically intractable epilepsy. Enhanced seizure susceptibility in rats due to genetic abnormalities (Clough et al. 1991) or experimental depletion of forebrain catecholamines (Barry et al. 1987, 1989) can be suppressed by intracerebral transplantation of fetal catecholamine-secreting neurons.

Evidence that rats can be protected from seizures by focal intracerebral microinfusion of drugs that mimic or augment the inhibitory action of gamma-aminobutyric acid (Gale and Iadarola 1980) raises the possibility that permanent protection may be provided by similarly placed transplants of fetal gamma-aminobutyric acid-secreting neurons, although a preliminary attempt has been unsuccessful (Stevens et al. 1988).

There are indications that fetal neural transplantation may have a future role in the treatment of traumatic injury and ischaemic or haemorrhagic injury (stroke) to the brain or spinal cord; at present, the consequences of these injuries are largely irreversible. Cortical neuronal degeneration after acute hypoxia in rats has been reported to be reduced by subsequent intracerebral implantation of fetal neocortical tissue (Polezhaev and Alexandrova 1984), and transplants of fetal hippocampal cells into the rat hippocampus one week after selective ischaemic hippocampal injury are able to re-establish anatomical and electrical features of the damaged local neuronal circuitry (Mudrick et al. 1989; Mudrick and Baimbridge 1991). This potential application of fetal neural transplantation is at present speculative.

Full functional recovery after spinal trauma or other massive central nervous system injury would require not only replacement of damaged nerve cells but reformation of long pathways and of the intricate neuronal interconnections necessary for normal sensory and motor function. Thus, potential application of fetal neural transplantation to such injury is speculative. Some progress has been made with respect to long-distance pathway reconstruction: nerve fibre regrowth through fetal brain and spinal cord tissues may be more extensive than through adult central nervous system tissue, and such fetal tissue transplants have been used to establish "bridges" for the reformation of long pathways in the lesioned adult rat central nervous system (Dunnett et al. 1989; Houle and Reier 1989). Implantation of other permissive substrates for long-range nerve fibre growth, such as peripheral nerve (David and Aguayo 1981), in conjunction with transplants of appropriate fetal neural tissue (Gage et al. 1985), may in the future facilitate the functional reconstruction of complex brain circuitry. Preliminary observations of functional effects of intraspinal implants of fetal monoaminergic brain tissue in rats with spinal cord lesions (Privat et al. 1988; Foster et al. 1990) raise the possibility of therapeutic applications of such fetal tissue transplants for treatment of paraplegia, to replace damaged descending monoaminergic projections (Tessler 1991).

Progress with respect to reconstruction of detailed microscopic patterns of connectivity has been less encouraging: no convincing demonstrations have shown reformation of ordered topographic connections in the damaged adult central nervous system by transplanted fetal neurons. Thus, the neurologic applications of fetal tissue transplantation most likely to be successful in the near future are those where damage or degeneration affects pathways in which precise spatial and temporal

patterns of neurochemical release are not essential for function. Notwithstanding these considerations, it has been claimed that cognitive impairments in rats after surgical removal of frontal neocortex can be reduced by transplantation of fetal cortical tissue to the lesion site (Labbe et al. 1983), and application of fetal brain tissue transplants to the treatment of cerebral palsies has been suggested (Rafael and Ayulo 1991).

Certain experimental neuroendocrine disorders, including diabetes insipidus (Gash et al. 1980), hypothalamic hypogonadism (Krieger et al. 1982), and pituitary hypothyroidism (Tulipan et al. 1985), have been treated successfully by transplants of fetal or neonatal hypothalamic or pituitary tissue in rodents. Although neuroendocrine disorders are generally responsive to replacement therapy with exogenous hormones, the possibility of physiologic feedback regulation of hormone release could lead to renewed interest in fetal neural transplantation for these disorders if the safety and efficacy of transplantation can be established.

Fetal tissue transplantation may find a future role in the management of chronic pain and of certain neuropsychiatric disorders. Transplants of adrenal medullary tissue — which contains, in addition to catecholamines, endogenous opioids known to influence central nervous system processing of pain — to pain-processing pathways in the spinal cord and midbrain have produced analgesia in rats (Sagen and Pappas 1987). Recently, allografts of adult cadaver adrenal medullary tissue to the spinal subarachnoid space has yielded long-lasting relief from chronic pain in terminally ill cancer patients (Sagen et al. 1991).

Advances in the understanding of the mechanisms of neuropsychiatric disorders may reveal neurochemical abnormalities that could be amenable to long-lasting correction by transplants of appropriate fetal neural tissue. This possibility is under investigation in the context of depression, in which central deficiencies of monoamine neurotransmission may play a role (Bryant and Brown 1986; Syvalahti 1987): preliminary observations suggest that intracerebral transplants of adult rat adrenal medulla and serotonin-secreting pineal tissue may prevent development of "learned helplessness," a widely studied model for depression, in rats (Sagen et al. 1990). According to a principal investigator in these transplant studies, human fetal tissue may be more effective than adult cadaver tissue for these applications (G. Pappas, pers. comm.), which at present are highly speculative.

Damage and degeneration of the retina have been treated experimentally in animals by transplantation of retinal pigment epithelial cells and strips of photoreceptor cell layer (Li and Turner 1988; Sheedlo et al. 1991b); histologic observations indicate that neonatal cells are more effective than adult cells in rescuing host photoreceptors from degeneration (Li and Turner 1991; Sheedlo et al. 1991a). Although the functional consequences of such grafts have not been ascertained, these observations suggest that transplantation of fetal human retinal cells may have future

applications in the treatment of retinitis pigmentosa, macular degeneration, and other retinopathies.

Certain myopathies may in the future benefit from fetal tissue transplantation. Transplanted myoblasts have been shown in mouse models of muscular dystrophy to fuse with degenerating muscle fibres, supplying sufficient normal genes or gene products to rescue the host muscle fibres (Partridge et al. 1989; Karpati et al. 1989). Over 30 boys suffering from Duchenne type muscular dystrophy — a progressive, fatal, X-linked degenerative disorder — have been treated by intramuscular allografts of adult donor-derived myoblasts. Structural and functional improvements after more than one year have been reported (Law et al. 1990, 1991), although these results are controversial (Thompson 1992b). According to the principal investigator of the study, human fetal myoblasts may be superior for transplantation to dystrophic muscle, and have already been used for this purpose in one patient in China (P.K. Law, pers. comm.).

Haematopoietic, Metabolic, and Genetic Disorders

Fetal liver tissue transplants may be considered for all applications where bone marrow transplants are currently used or contemplated. In addition to the various forms of severe combined immunodeficiency, combined immunodeficiency, and other primary haematopoietic disorders described earlier, additional haematopoietic disorders that could in the future prove amenable to these transplants include AIDS and inherited disorders, such as Fanconi's anaemia, Wiskott-Aldrich syndrome, chronic granulomatous disease, Kostmann's syndrome, Chédiak-Higashi syndrome, Maroteaux-Lamy syndrome, infantile malignant osteopetrosis, agammaglobulinaemia, and sickle cell anaemia.

The possibility of restoring haematopoietic function that has been depleted by anti-cancer therapy opens a potentially vast range of applications for human fetal liver tissue transplantation. The failure of anti-cancer therapy reflects the ability of cancer cells to escape the effects of medication or irradiation, the doses of which are limited by unavoidable damage to healthy proliferating cells of the patient's body, in particular the haematopoietic cells of the bone marrow. This limitation could in principle be circumvented by the use of fetal haematopoietic cell transplants after the administration of higher, toxic doses of the anti-neoplastic agents. Thus, in addition to leukemias, such fetal liver tissue transplants may find applications in the treatment of major causes of death in Canada, such as breast cancer.

As indicated previously, fetal haematopoietic cell transplants can serve to resupply not only missing elements of the blood and immune system, but gene products missing from other tissues (Karson and Anderson 1990; Johnson et al. 1989). Such applications face serious problems regarding the targeting of these gene products, particularly structural proteins, to the appropriate cell; nevertheless, there are indications from animal experiments that grafted haematopoietic stem cells could significantly

restore enzyme levels in the central nervous system in lysosomal storage disorders (Taylor et al. 1986). Genetic disorders that could potentially benefit from this approach include, in addition to those mentioned previously, a wide range of relatively uncommon diseases such as Lesch-Nyhan syndrome, Tay-Sachs disease, Sandhoff disease and other lipidoses and mucopolysaccharidoses, ornithine transcarbamoylase deficiency, and complement clotting, and other factor deficiencies such as haemophilia. The ability of fetal haematopoietic stem cells to provide gene therapy may in the future be greatly increased by the insertion of exogenous genes into these highly proliferative cells; the feasibility of this approach has been demonstrated in animal experiments by several authors (Kantoff et al. 1989; Karson and Anderson 1990; Crombleholme et al. 1990, 1991).

Transplants of fetal liver tissue may also be of use in the future for correction of certain consequences of congenital or acquired hepatic dysfunction. Since hepatic cells, rather than the earlier-developing haematopoietic stem cells, are needed for these purposes, tissue from fetuses of more than 12 weeks' gestational age may be most useful for the treatment of such disorders as congenital hypoalbuminaemia, biliary atresia, and cholestatic syndromes. These and other fatal disorders — including end-stage renal disease and severe congenital heart diseases, such as hypoplastic left-heart syndrome — that afflict several hundred children each year in North America may be treatable by fetal whole organ transplantation (Flake et al. 1986; Verrier et al. 1989; Drugan et al. 1989; Crombleholme and Harrison 1990). However, availability of late-gestational organs suitable for such transplantation is limited, and the most likely source is the anencephalic infant. Kidneys have been transplanted from anencephalic infants since 1961 (Goodwin et al. 1963), and up to 1984 at least 23 transplants of kidneys from anencephalic donors had been performed around the world, with six kidneys functioning for more than one year (Itatka et al. 1978; Kinnaert et al. 1984); however, the use of anencephalic tissue (Cefalo and Engelhardt 1989; Zaner 1989; Walters 1991) is beyond the scope of this review and will not be considered further. Alternatively, dissociated fetal hepatic tissue may be transplanted to ectopic sites such as the spleen (Nozawa et al. 1991) or incorporated into synthetic "neo-organs" (Newmark 1989) to supply the patient with missing liver-derived substances. It is possible that such an approach may prove to be of value in the treatment of acquired hepatic insufficiency due, for example, to chronic alcoholism or viral hepatitis.

Creation of New Biomedical Tools

Animal models of human disease, involving grafts of fetal human tissue to immunodeficient host animals, are already in use; they may be used increasingly in the future to study the development, function, and pathologies of various human organs and systems *in vivo* in those contexts where grafts of adult tissues (Cannon et al. 1990) cannot be used, for

example, to study the dissemination of human neurotropic viruses. Such heterochimeric animal models may also be of value in identifying, developing, and testing new chemotherapeutic and immunologic treatments that would be difficult, impractical, or unethical to study in humans.

Heterochimeric models can be used for the production of human polyclonal or monoclonal antibodies for therapeutic purposes (e.g., for construction of immunotoxins for the treatment of cancer) (Duchosal et al. 1992). Poisons coupled to tumour-specific antibodies may greatly increase the efficacy and safety of anti-cancer therapy; however, antibodies raised in non-human species are recognized as foreign and may be eliminated before they reach their intended targets. Human anti-tumour antibodies, raised in heterochimeras, may provide a solution to this problem.

The successful development of these tools will lead to commercial applications. At least one company in the United States is already pursuing this possibility. According to its founder and scientific director, SyStemix, Inc., of Palo Alto, California, does not plan to sell the chimeric organisms it produces containing grafted human fetal tissue (J.M. McCune, pers. comm.). However, contractual research using such organisms, or sale of reagents such as human antibodies derived from them, can be expected.

Implications

The range of current and future applications of human fetal cadaver tissue outlined in the previous sections gives evidence of the importance of these applications for the advancement of knowledge and the improvement of medical therapy. However, increasing use of this tissue will also have ethical, legal, and social implications.

Ethical and Legal Issues

The complex ethical and legal issues raised by the use of human fetal tissue have been extensively reviewed (McCullagh 1987; Mahowald et al. 1987a; Lewin 1987; Fine 1988; Nolan 1988; Freedman 1988; Jonsen 1988; U.S. National Institutes of Health 1988a; Gillam 1989; Dickens 1989; Gold and Lehrman 1989; Rosner et al. 1989; Robertson 1990; Jones 1991). The detailed and balanced presentations in the *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 1 (U.S. National Institutes of Health 1988b), in *Neural Grafting: Repairing the Brain and Spinal Cord* (U.S. Congress 1990), and in *The Use of Human Fetal Tissue: Scientific, Ethical, and Policy Concerns* (Vawter et al. 1990) are conspicuously valuable. The following observations are offered from a pragmatic, utilitarian perspective.

Serious objections to the use of human fetal cadaver tissue mainly arise from the origin of such tissue in the termination of pregnancy. An important objection is the contention that use of human fetal cadaver

tissue will lead to inducements or incentives for women to undergo abortion, influencing their decision and increasing the number of abortions performed. At present, no empirical evidence supports this claim. It is also plausible that the incidence of abortion may be decreased by certain uses of human fetal cadaver tissue, such as transplantation *in utero* to treat fatal congenital disorders in fetuses that otherwise would be aborted. No reports of dramatic changes in the incidence of abortion have come from countries or locales where well-publicized clinical trials of human fetal tissue transplantations have begun. Possible effects of future use of human fetal cadaver tissue on abortion incidence cannot be predicted, but would appear not to constitute reasonable grounds for prohibiting this tissue use at present. Empirical data bearing on this and other implications of human fetal cadaver tissue use are urgently needed. (Tuch et al. (1990), in an initial step toward supplying empirical data bearing on human fetal tissue use, surveyed researchers handling this tissue to address the possibility that such exposure might "brutalize" or in other ways alter their ethical views or behaviour; their results offered no support for this possibility.)

The potential that fetal tissue use would encourage women's decisions to terminate pregnancy could be reduced in several ways. It could be required that consent for tissue use be requested only *after* consent has been obtained for the abortion. In addition, the targeting of fetal tissue to specific recipients — a practice that would appear to be permissible under present tissue donation laws in Canada (Dickens 1989) — could be prohibited.

On the other hand, some have argued that a woman should be entitled to become pregnant with the aim of aborting to obtain fetal tissue for transplantation to herself or to a family member. According to this view, the legitimate interests of that family member and of the woman take precedence over those of the fetus, which though human is not yet a person (Warren 1978; Robertson 1988b; Brody 1990). In addition to potential cases (Lewin 1987), at least one actual case appears in the medical literature in which a pregnancy seems to have been terminated expressly to provide fetal tissue for transplantation (Kelemen 1973): this was in Hungary, where a patient with aplastic pancytopenia underwent abortion, after which she received a transplant of liver tissue derived from her six-to-seven-week-gestational-age fetus. The patient was in remission after 19 months, but no evidence of engraftment was obtained. Ethical issues were not discussed in the report of that case. (It is worth noting that for treatment of diseases with autoimmune components, perhaps including insulin-dependent diabetes mellitus and multiple sclerosis, transplants of closely related tissue may be particularly *unsuitable*.)

Whether or not abortion — or conception and abortion — with the intent of providing fetal tissue for transplantation or other purposes is intrinsically immoral, many people are "very uncomfortable with the idea of intentionally using conception and pregnancy to produce anything other than babies" (Greely and Raffin 1989). However, attempts to police

women's intentions, as in proposed U.S. government regulations such as the National Institutes of Health (NIH) Revitalization Amendments of 1991 requiring women to certify that they are not terminating their pregnancy with the aim of providing fetal tissue, not only would be unreliable but would constitute undesirable violations of privacy (Kearney et al. 1991).

The unavoidable uncertainties of fetal tissue recovery provide another disincentive to abortion for targeted donation: first-trimester abortions, performed using routine vacuum technique, invariably fragment the fetus, with the result that particular organs for transplantation can be retrieved in only a fraction of cases.

Objections that use of human fetal cadaver tissue exploits the fetus, treating it only as means to an end, lose their force when the abortion that makes this tissue available is performed for reasons unrelated to the tissue use. However, some commentators who consider abortion — whether legal or not — to be immoral have objected to the use of tissue derived from abortions on grounds of moral complicity. In some cases, this argument has been made by analogy with research on concentration camp inmates in Nazi Germany (Anderson 1988; Burtchaell 1988; Bopp and Burtchaell 1988). In response, it has been argued that these objections are mistaken if, as a large segment of the population believes, abortion is not immoral, and are furthermore misleading on two crucial points (Robertson 1988a, 1990). First, moral misdeeds may be foreseen without being intended (Miller 1989). For example, the use of fetal cadaver tissue no more entails complicity in abortion than use of cadaver organs from a homicide or an automobile accident entails complicity in murder or drunk driving. Second, the immoral Nazi concentration camp experiments harmed living people, but the dead fetus cannot be harmed by use of its tissues. No doctors were prosecuted at the Nuremberg trials for use of cadaver tissue derived from concentration camp experiments.

For the use of human fetal cadaver tissue to be acceptable, tissue must be obtained by legal means from legally performed abortions (Robertson 1990). In Canada, unlike the United States, there at present appears to be no legal requirement for the woman undergoing abortion to consent to research or therapeutic use of removed tissue (Dickens 1989). Indeed, some have suggested that the woman's consent is inappropriate, since she cannot, by virtue of her abortion, be considered a proxy acting in the interest of the fetus (Bopp and Burtchaell 1988). However, as the woman may have interests of her own in the uses of the fetal tissue, particularly where fetal cells may be maintained in a viable state in transplants or in tissue culture, it would seem reasonable and appropriate to require her consent for such uses.

Some writers have suggested that it may be appropriate to prolong a pregnancy or to alter the method of abortion "in order to increase the chances of therapeutic success for the recipient" (Mahowald et al. 1987a). Researchers in Sweden have already modified standard first-trimester vacuum abortion techniques "to obtain less damaged fetal tissue" (Olson

et al. 1987), using forceps (*ibid.*) or a manual syringe under ultrasound guidance (Gustavii 1989). The modified abortion procedures appear to have been used to obtain fetal brain tissue for transplantation to Parkinson's disease patients (*ibid.*), notwithstanding statements that transplanted tissue was obtained from "routine suction abortions" (Lindvall et al. 1990) and that "the collection of tissue for transplantation (in Sweden) in no way influenced the abortion procedures" (Gunning 1990).

In the United States, a modified vacuum abortion technique used specifically to obtain human fetal tissue for research is reported "to extract intact first-trimester fetuses 80 percent of the time" (Kolata 1989). The particular modifications may impose no additional risk upon the pregnant woman (although this has not been documented), but they seem to undermine the separation between the abortion and the uses of the aborted fetal tissue, the importance of which has been emphasized above.

The modification of procedures so that they lead to recovery of largely intact fetuses raises additional legal and ethical problems related to determination of fetal death. Brain death criteria used for adults are considered inapplicable to the early fetus (Dickens 1989). Guidelines or legislation establishing appropriate criteria for fetal death are lacking and urgently needed; at present and in their absence, the cessation of heartbeat and spontaneous movements in an intact fetus appears to be taken as the signal for commencing tissue dissection. However, use of tissue from a fetus deemed not dead when removed from the woman's body would be restricted in Canada by existing laws on post-mortem donation, and could by law be vetoed by either parent (*ibid.*). There is currently no obvious means of determining compliance with such laws.

There is justified concern that commerce in fetal tissue could result in financial incentives for abortion. Kidneys for transplantation from live donors in Brazil and India have reportedly been advertised for sale to physicians in Germany (MacKenzie 1985). An impoverished Turkish donor was paid US\$3 300 to provide a kidney for transplantation in Britain, and a German-based business has reportedly offered European donors US\$30 000 to \$50 000 for transplantable kidneys (Trucco 1989). It has been suggested that the sale of organs for transplantation could be permitted if it is necessary to increase their supply (Childress 1989; Hansmann 1989; Trucco 1989). Lurid reports of children in India and Thailand sold into prostitution even before birth have been invoked to raise the spectre that women in developing countries "will be bought as breeders" (Raymond 1989).

However, partly in response to reports such as these, Canada, the United States, and most of Western Europe have passed laws prohibiting the sale of human organs. Such laws could be amended to include fetal cadaver tissue. There is no reason to believe that commerce in fetal tissue, financial incentives for abortion, prolongation of gestation before abortion, or other procedural alterations not in the medical interest of the pregnant

woman could not be effectively prohibited or otherwise controlled by legislation (Dickens 1993).

A request for approval to initiate a clinical trial of human fetal neural tissue transplantation for the treatment of Parkinson's disease at the U.S. NIH resulted in the imposition in 1988 by the Department of Health and Human Services of a moratorium prohibiting federal funding of human fetal tissue transplantation research using tissue from induced abortions. It also resulted in the convening of an advisory committee and an ad hoc panel of consultants to examine scientific, ethical, and legal issues in the use of human fetal tissue for medical research. After public hearings, and on the basis of the panel's report (U.S. National Institutes of Health 1988b), the advisory committee later that year recommended unanimously that the moratorium be lifted (U.S. National Institutes of Health 1988a). As of April 1992, these recommendations have not been followed, and the moratorium remains in effect. The continued moratorium is reported to have "had the secondary effect of discouraging basic research in neural grafting" and other types of human fetal tissue transplantation (U.S. Congress 1990). At the same time, privately funded clinical trials of fetal neural transplantation in Parkinson's disease, begun in spite of the moratorium by investigators at the University of Colorado and at Yale University, have been criticized as creating an "impression of the illegitimacy of the federal process" (Annas and Elias 1989b).

Obtaining Tissue

Current Sources of Tissue

Because of considerations of viability, health, and availability of tissue, most human fetal cadaver tissue used for research is collected from induced abortions. Almost all of these procedures are performed between the fifth and twelfth weeks of gestation, by intrauterine suction techniques. Fetal tissue from these procedures, although fragmented, is generally viable, and can be collected without contamination; as outlined in previous sections, tissues at these stages of development are satisfactory for many applications, and optimal for some. Termination of later pregnancies is less common, and is performed mainly by other procedures, chiefly prostaglandin administration or saline infusion, that yield less-viable fetal tissue (Wong 1988). To obtain viable tissue from second-trimester abortions, it seems to be necessary to use a dilation and evacuation technique that may be safer than other methods but only when used by doctors performing the operation frequently (Kolata 1989).

In Canada, human fetal cadaver tissue for research is obtained by investigators directly from facilities where pregnancy termination is carried out or, in some cases, from other investigators who are the direct recipients; no payments are involved, except for transportation of tissue. In Great Britain, the major supplier of human fetal cadaver tissue to researchers has, since its inception in 1957, been the central Medical

Research Council Tissue Bank in London, which receives tissue without payment from London area gynaecologists; over 4 000 tissue specimens up to 20 weeks' gestational age (but mainly first trimester) derived from approximately 2 500 abortions are distributed annually to approved investigators, with no payment except transportation costs (Wong 1988; Vawter et al. 1990).

In the United States, it has been asserted that most investigators obtain fetal cadaver tissue through private arrangements with local abortion clinics (Kolata 1989; Vawter et al. 1990). Some of these clinics, members of the National Abortion Federation, comply with guidelines requiring separate patient consent to the abortion procedure and to the use of fetal tissue for research; no financial compensation goes to the patients, and no financial profit is gained by the clinics. Two clinics supplying tissue to for-profit corporate laboratories have received reimbursement from those corporations for their administrative costs (National Abortion Federation, pers. comm.). No information is available regarding arrangements between researchers and other non-member gynaecologists or abortion clinics in the United States.

Alternatively, investigators in the United States can obtain human fetal cadaver tissue from centralized tissue distribution agencies. These agencies, like the British Medical Research Council Tissue Bank, serve as intermediaries maintaining donor and recipient anonymity. The IIAM, a non-profit tissue procurement agency in Exton, Pennsylvania, is currently the largest U.S. supplier of human fetal cadaver tissue, providing specimens to investigators upon submission of written request. Ethical review board approval is required of academic researchers; investigators at for-profit corporation laboratories are asked to document corporate approval of the proposed research. Since its inception in 1986, IIAM has supplied fetal tissue to 67 non-profit institutions, including the U.S. NIH (in three cases for development of clinical transplantation therapies), and to 19 for-profit corporations (in three cases for development of transplantation therapies). Neither patients nor physicians performing the abortions are paid by IIAM for providing tissue, but the abortion clinics are reimbursed for supplies and use of facilities; investigators are charged a fee-for-service of US\$65 per fetal tissue sample, or \$180 if sterile tissue is required (B.M. Bardsley, pers. comm.).

In 1989, *The New York Times* reported that IIAM "encourages doctors ... to use special suction abortion techniques ... to get usable tissue" (Kolata 1989); the 80 percent recovery of intact fetuses cited previously was obtained by a doctor providing tissue to IIAM. According to its executive director for research services, IIAM no longer recommends modification of abortion procedures. Instead, it obtains tissue from a small number of clinics that use the special suction abortion techniques routinely (B.M. Bardsley, pers. comm.).

A second U.S. human fetal tissue distribution agency is the Laboratory of Human Embryology in the Department of Pediatrics, University of

Washington. This laboratory has collected over 10 000 specimens of human fetal cadaver tissue, mostly from first-trimester abortions in Seattle area clinics, since 1961. Tissue has been distributed to about 50 requesting investigators per year, at institutional and for-profit laboratories, without payment from investigators or to abortion clinics; approval of requests by ethical review board has not been required (A. Fantel, pers. comm.).

Advanced Bioscience Resources, Inc., a third centralized distribution agency, is a for-profit company based in California. This company collects human fetal cadaver tissue from private abortion clinics and provides specimens to academic investigators and, mainly, to corporate laboratories, for undisclosed fees (L. Tracy, pers. comm.). A fourth agency, the National Disease Research Interchange, is a NIH contract-supported organization that supplied up to 3 000 human fetal cadaver tissue samples each year to some 23 investigators between 1984 and 1987, when tissue collection was discontinued on the recommendation of NIH advisors. Collection will not recommence while the moratorium on federal support for fetal tissue transplantation remains in force in the United States (Ducat 1988).

Commercial Involvement in Fetal Tissue Procurement

Commercial considerations in human fetal cadaver tissue procurement are present at several levels. As described in the preceding section, non-profit tissue procurement agencies may charge researchers a fee-for-service to obtain human fetal tissue, and they may reimburse abortion clinics for costs incurred in tissue collection. At least one for-profit company, Advanced Bioscience Resources, Inc., currently collects human fetal cadaver tissue in the United States, but details of the operation of this private company are not in the public record, and requests for information for this report were refused. Another U.S. for-profit company, Hana Biologics, obtained human fetal cadaver tissue from IIAM, and proposed to profit through the sale of proliferated, safety-tested, quality-controlled cells of human fetal origin to surgeons and investigators for therapeutic and research applications (Voss 1988). Hana Biologics is also the only documented commercial participant in clinical transplantation of human fetal tissue. Over 30 fetal pancreas transplants in the United States were performed under its sponsorship (*ibid.*; Highfield 1988). Hana Biologics no longer exists as an independent entity, having been acquired by Somatics Therapeutics, a Boston and Alameda-based biotechnology company, during corporate restructuring. This structuring also involved merger with Genesis Therapeutics of La Jolla, California (H.F. Voss, former vice-president Hana Biologics, pers. comm.). The reorganized company has discontinued Hana's transplantation work, and now has "zero interest in human fetal tissue" according to a company executive (H. Hickson, associate director Somatics Therapeutics, pers. comm.). Accounts of international commerce in human fetal tissue were related previously.

Alternatives to the Use of Tissue from Induced Abortions

Many of the ethical and legal issues raised by transplantation or other uses of tissue derived from induced abortions could in principle be avoided by use of alternative material. Even if these issues were resolved, the development of alternative tissue sources would still be of value to avoid problems of availability and disease transmission potentially associated with the use of human fetal tissue. As summarized below, currently available alternatives are generally less satisfactory than aborted material for many applications, particularly transplantation. The development of more useful alternatives will be important; unfortunately, despite intensive current research in this area, the advent of such alternatives for clinical use may be years away.

There are indications that spontaneous abortions could be a limited source of useful tissue, particularly from late-gestation fetuses (Thorne and Michejda 1989). However, the high incidence of chromosomal abnormalities (Warburton et al. 1980; Huisjes 1984) and contamination (Sever 1980) in tissue from spontaneously aborted fetuses renders this material largely unsuitable for human transplantation and most other applications (Ducat 1988; Annas and Elias 1989a). In the United States, spontaneous abortions among a population of 10 000 pregnant women would yield no more than 14 eighth-to-sixteenth-week-gestation fetuses annually, suitable as sources of tissue for transplantation, at a cost of US\$3 million for collection and screening (Kline et al. 1992). Surgical removal of the fetus as treatment of life-threatening ectopic pregnancies could yield normal and thus more useful fetal cadaver tissue (Nolan 1988). However, the low incidence and unpredictable occurrence of ectopic pregnancy restrict the availability and usefulness of this source of tissue (although see the discussion of cryopreservation, below). The advent of non-surgical treatment for ectopic pregnancy (Tulandi 1991) also is likely to reduce further the availability of tissue from this source.

Umbilical cord blood, obtained from normal placenta at the time of birth (Auerbach et al. 1990) or during fetoscopy (Rayfield et al. 1987), is a relatively abundant source of multi-potential haematopoietic stem cells (Nakahata and Ogawa 1982) that may be useful for transplantation (Broxmeyer et al. 1989). At least two children with Fanconi's anaemia, a rare inherited pancytopenia, have been treated by transplantation of cord blood from normal HLA-matched siblings (Gluckman et al. 1989; Auerbach et al. 1990) with evidence of engraftment (Broxmeyer et al. 1990). Thus, umbilical cord blood may be a useful alternative or adjunct to fetal liver for transplantation for particular disorders. However, the concentration of multi-potential stem cells can be expected to be significantly higher in first-trimester fetal liver than in umbilical cord blood at birth. Also, the greater immunologic maturation of cells in cord blood will, at least in the near future, restrict its transplantation to closely matched recipients.

Human fetal cadaver tissue can remain viable after deep freezing and thawing (Stenchever et al. 1965; Farkas et al. 1990), and such

cryopreserved tissue has already been used for clinical transplantation, although available results are limited and disappointing (Dawidson et al. 1988; Redmond et al. 1990). The development of better cryopreservation procedures appears feasible, and could increase the usefulness of unpredictable sources of fetal tissue, e.g., ectopic pregnancies, as adjuncts to the use of tissue from elective abortion. The creation of "tissue banks" of such stored material could also help to further the separation between abortion and the subsequent use of fetal cadaver tissue, and would facilitate rigorous safety testing of tissue before its use. Shorter-term culture or refrigerated (unfrozen) storage of fetal tissue may also achieve some of these objectives, with less loss of cell viability (Kawamoto and Barrett 1986).

Transplants of adult adrenal medulla cells can survive within the brain and can produce functional improvements in experimentally parkinsonian rats (Freed et al. 1981). Despite observations that grafts of fetal dopaminergic brain tissue are more effective than grafts of adult adrenal medulla in correcting parkinsonian symptoms in animals (Freed 1983; Bakay and Herring 1989; Gash et al. 1991; Dunnett and Annett 1991; Bakay 1991), clinical trials of intracerebral adrenal medullary transplantation were initiated for treatment of Parkinson's disease (Backlund et al. 1985), largely to avoid controversy associated with the use of fetal tissue. After publication in the influential *New England Journal of Medicine* of claims of substantial improvement in parkinsonian symptoms after adrenal medullary autografts (Madrazo et al. 1987), many such procedures were performed around the world. These operations have generally had disappointing results (Fine 1990; Gash et al. 1991). The required adrenalectomy operations have been associated with high morbidity and mortality (Madrazo et al. 1990b). In most centres, adrenal medullary grafts have been discontinued, and attention has returned to the use of fetal brain tissue. However, the functional effects of adult adrenal medulla grafts may possibly be augmented in the future through treatment of the medullary tissue with appropriate trophic factors (Stromberg et al. 1985; Fine 1990).

Many of the problems associated with human fetal tissue use could be avoided by use of non-human fetal tissue. Indeed, such material is widely used for research that does not strictly require human tissue. Large-scale production of fetal animal pancreatic islets and other tissue is possible (Korsgren et al. 1988), and their use for clinical transplantation has been suggested. However, species differences may limit the usefulness of this approach. For example, fetal neurons from rodents are less able than human fetal neurons to grow fibres over long distances, and thus may be less able to reinnervate the relatively larger volumes of human brain targets (Stromberg et al. 1989; Wictorin et al. 1990; Fawcett 1992). Furthermore, because all cells, even those lacking major histocompatibility antigens, express species-specific antigens that can be recognized as foreign, survival of tissue from other species (xenografts) generally requires protection from

rejection by the host immune system. Xenografts of fetal neural and adrenal tissue into the brain of experimental animals can survive for some months (Kamo et al. 1987; Perlow et al. 1980), but large and maintained doses of immunosuppressive medications are generally needed for them to manifest persistent functional effects (Brundin et al. 1985). Xenografts have been considered for clinical application (Huffaker et al. 1989), but potential toxic effects of prolonged use of cyclosporin and other currently available immunosuppressives have discouraged pursuit of this approach. Development of safer and more effective methods for patient immunosuppression (e.g., targeted selectively against transplant antigen-specific lymphocytes) (Lenschow et al. 1992) could increase the usefulness of xenografts, and could permit the use of mismatched post-natal cadaver tissue for certain applications such as pancreatic islet cell transplantation. It should be noted that the killing of animals to provide tissue for clinical transplantation is not without ethical difficulties, and cogent arguments have been made against this practice (Nelson 1992).

Methods to decrease antigenicity of graft tissue, such as shielding of antigens by antibodies, may also be useful in this way. Microencapsulation of fetal human islets for allografting to insulin-dependent diabetes mellitus patients has already been described, but this same technique may be an effective alternative to immunosuppression for preventing rejection of xenografts. Encapsulated fetal (Krestow et al. 1991) and adult (Lacy et al. 1991) rat islet xenografts have been shown to survive and restore normoglycaemia in diabetic mice, and encapsulated bovine adrenal medullary cells have brought about partial reductions in abnormal locomotion after intrastriatal xenografting in a rat experimental model of parkinsonism (Aebischer et al. 1991). This approach may prove useful for transplantation therapy of endocrine or metabolic disorders, but is not likely to be useful for neurologic or other applications where direct contact between graft and host cells is important.

Continuously propagated cultures (clonal cell lines) have obvious advantages as a potentially limitless source of tissue for transplantation. However, since central nervous system neurons do not normally proliferate, such lines must currently be obtained from transformed or tumour-derived cells. Even after differentiation-inducing treatments, transplants of such cells can give rise to tumours upon transplantation (Jaeger 1985; Gash 1988; Freed et al. 1989; Allen and Kershaw 1989). In addition, transformed cells may express abnormal antigens on their surfaces that can be recognized as foreign, leading to their rejection in the absence of immunosuppression (Freed et al. 1986). Such rejection, and the potential spread of cancerous cells, may in the future be avoided by implantation of transformed cells encapsulated within semi-permeable membranes (Winn et al. 1991). However, this will be subject to the considerations outlined above. Other approaches to the propagation of non-cancerous lines of human fetal or adult endocrine, neuronal, or multi-potent stem cells by various means, including transfection with inducible oncogenes and treatment with various

growth factors, are under development ("Immortal Cells" 1990; Fine 1990; Hisanaga et al. 1991). Recently, proliferating cells in the adult mouse brain have been shown to be capable of generating neurons and astrocytes (Reynolds and Weiss 1992). Such cells could in principle provide an alternative source of tissue for transplantation. However, their presence in the human brain, their proliferative capacity, and the range of neuronal subtypes they could generate have yet to be established. Other related possibilities include the routine tissue banking of cryopreserved multi-potent stem cells by healthy individuals at an early age, for transplantation in the event of their later illness (Golde 1991).

Research is currently under way to identify endogenous compounds or drugs that will stimulate restoration of function by the patient's own body, thus eliminating the need for tissue transplantation. Production of blood cells, for example, may be augmented by use of various cytokines, erythropoietin, or other factors, while survival and fibre regrowth of injured neurons may be enhanced by administration of neurotrophic factors, thus avoiding need for exogenous cells. However, such applications for cell and tissue transplantation likely will remain, and these trophic factors will probably be used as adjuvants to enhance the effectiveness of transplantation therapies.

Deficient hormones or neurotransmitter substances may be supplied by genetic manipulation of the patient's own cells, either *in situ* by retroviral vectors or direct deoxyribonucleic acid injection (Rojas and Hoffman 1991), or *ex vivo* followed by reimplantation (Karson and Anderson 1990). The feasibility of the latter approach is suggested by the ability of rat fibroblasts, transfected with the gene for tyrosine hydroxylase (the enzyme catalyzing dopa production from tyrosine), to ameliorate abnormal locomotion after intrastriatal transplantation into experimental parkinsonian rats (Wolff et al. 1989). As indicated previously, such gene therapy may be of use in the treatment of certain inborn errors of metabolism and other diseases resulting from single-gene defects. For other purposes, these approaches will, in their simplest forms, be less effective than fetal tissue transplantation. Thus, for example, restoration of normal neurologic function may require not only the synthesis of a particular neurotransmitter substance, but also the cellular machinery for packaging and physiologically regulated release of that substance, directed fibre outgrowth, and the formation of appropriate synapses. Alternatively, genetic engineering methods may be used to provide a chronic source of adjuvant trophic factors; thus, intracerebral grafts of rat fibroblasts transfected with the gene for nerve growth factor have enhanced the metabolism or survival of intrinsic and grafted acetylcholine neurons (Rosenberg et al. 1988; Ernfors et al. 1989).

Supply and Demand Considerations

Demand for human fetal cadaver tissue for research and therapeutic uses in Canada and the United States corresponds at present to a very small fraction of the number of abortions performed. Fetal tissue is currently collected from about 1 percent of abortions performed annually in Canadian hospitals (A. Fine, unpublished observations). In the United States, the collection rate has been estimated to be 0.2 percent (Ducat 1988). More than 90 percent of women undergoing abortion will likely consent to the use of tissue for research or transplantation (Olson 1988; Vawter et al. 1990; A. Fine, unpublished observations), so that, in principle, fetal tissue could be collected from most of the 65 000 or so hospital abortions performed annually in Canada (Rauhala 1988).

Comparison of different laboratories' rates of recovery of particular tissues from the collected fetal remains suggests that recovery is highly dependent on the experience of the technician, on the particular tissue sought, and on the timing and method of abortion (Markowski and Lawler 1977; Wong 1988; I. Stromberg, Karolinska Institute research scientist, pers. comm.; A. Fine, unpublished observations). For example, with routine vacuum abortions in the first trimester, fetal dopaminergic brain tissue suitable for use in Parkinson's disease can be recovered from approximately 30 percent of collected fetal remains; fetal liver suitable for use in haematopoietic reconstitution, metabolic, and genetic disorders can be recovered in at least 20 percent of cases; and fetal striatal brain tissue suitable for use in Huntington's disease can be recovered in at least 5 percent of cases (A. Fine, unpublished observations). Recovery of fetal pancreas and thymus from routine first-trimester material is more difficult, but they are relatively easily dissected from the more intact fetal remains from second-trimester abortions and modified low-vacuum abortions. These recovery rates are independent: more than one organ or type of tissue can be recovered from the remains of a single fetus.

This potential availability of tissue can be compared with estimated annual incidence in Canada of those diseases most likely to be treatable by human fetal tissue transplantation in the near future. Approximately 8 000 new cases of Parkinson's disease occur in Canada each year, while the annual Canadian incidence of insulin-dependent diabetes mellitus is approximately 4 000; of all leukemias and congenital immunodeficiency disorders, approximately 4 000; of Huntington's disease, approximately 100 (statistics derived from data of Health and Welfare Canada, U.S. Congress Office of Technology Assessment, Parkinson Foundation of Canada, Canadian Diabetes Association, and Huntington Society of Canada). Most Parkinson patients will respond to medical therapy; in the near future it is extremely unlikely that more than several hundred Parkinson patients would be treated annually in Canada by fetal neural transplantation. Induced abortions in Canada could, with skilled and efficient tissue collection, easily sustain this, even if tissue from as many as 20 fetal brains were required for each patient. These figures suggest that the availability

of human fetal cadaver tissue in Canada is sufficient to meet realistic foreseeable needs in the near future.

Longer-range predictions of supply and demand are complicated by important uncertainties, and are probably unwarranted. The efficacy and safety of other potential applications of fetal tissue are unknown. Although some of these applications could involve much larger numbers of patients, the development of preventive measures, alternative therapies, or alternative sources of transplantable tissue could eliminate many potential future needs for human fetal cadaver tissue. At the same time, changes in the incidence of abortion, due, for example, to changes in legislation, public values and perceptions, or increased availability of abortifacient drugs such as RU-486, could affect in unpredictable ways the availability of suitable tissue for transplantation. Any policy decisions involving considerations of supply and demand for human fetal cadaver tissue will require periodic re-evaluation in light of such changes.

Public Policy: Issues and Recommendations

The use of human fetal cadaver tissue raises issues whose resolution may require the establishment of guidelines, legislation, and regulatory bodies.

Some of these issues pertain to medical experimentation and surgical innovation in general. For example, no uniform criteria have been developed for deciding when it is appropriate to apply a new surgical technique to patients, or whether and when it may be necessary first to study the effects of new procedures on animals, particularly subhuman primates. Appropriate agencies for establishing these criteria have not been designated or established nationally. Approval of new techniques is largely under the control of local institutional review boards or professional licensing and accreditation boards. In turn, no uniform standards exist for accreditation and evaluation of institutional review boards in Canada. In addition, when experimental techniques disseminate, there are no uniform vehicles or criteria for evaluating and approving the safety and efficacy of their implementation by particular hospitals and physicians (e.g., whether their outcome after a defined period is superior to that at other approved centres or with other methods).

Other issues pertain more specifically to the use of human fetal cadaver tissue. At present, Canada lacks legislation explicitly defining conditions under which abortion is permitted. Conditions under which tissue from legal abortions may be used for research, therapy, or other applications are also not defined by law. There are no guidelines, criteria, or vehicles for the approval of individual physicians or clinics as sources of fetal cadaver tissue. The ethical, safety, technical, and record-keeping standards of such agencies as the National Disease Research Interchange in the United States and the Medical Research Council Tissue Bank in Great Britain suggest that the benefits of government-supported and

regulated centralized organizations for collection and distribution of human fetal cadaver tissue would be better than the unregulated and undocumented individual agreements with local abortion clinics by which Canadian researchers currently obtain such tissue. The suitability of commercial involvement in such distribution should be determined, if for-profit companies could provide distribution, serologic and microbiologic testing of tissue, or other essential services less expensively than government or hospital facilities.

There are at present no definitive legislative or judicial directives regarding requirements for consent of either parent of the aborted fetus for use of its tissues. There are no uniform guidelines or legislation with respect to the permissibility of non-harmful modification of abortion procedures expressly to facilitate recovery of fetal tissue. There are no appropriate criteria for establishing fetal death at different developmental stages. There is no uniform standard or central data base to keep records on the collection or distribution of human fetal cadaver tissue, or the purposes and results of its use.

The federal and provincial governments have not made explicit the conditions, if any, under which they will pay for research and therapeutic uses of human fetal cadaver tissue. The lack of such support in Canada can be expected to inhibit the relevant areas of medical progress, and to deny access to fetal tissue transplantation procedures to those unable to seek them abroad. Government support of such research could furthermore provide a basis for record keeping and the establishment of medical, scientific, and ethical standards pertaining to human fetal cadaver tissue use. The costs of therapeutic uses of human fetal cadaver tissue will vary, depending on the specific procedures and the hospital where they are performed. In the United States, the cost of clinical trial of fetal neuronal transplantation for Parkinson's disease has been estimated at US\$100 000 per patient (Donovan 1990). The cost of islet cell transplantation for insulin-dependent diabetes mellitus is likely to be similar, but these costs would likely fall precipitously if these procedures became routine. In Canada, the cost per patient of clinical trial of fetal neuronal transplantation for Parkinson's disease is significantly lower — approximately C\$25 000 — in large part because of the donation of investigators' labour; this procedure can in principle be performed on an outpatient basis, and its cost, once routine, might be as low as one-fifth of its current cost in clinical trial (A. Fine, unpublished observations). Annual costs of some of the potentially treatable diseases — including direct medical care, lost productivity, and other factors — have been estimated; for example, it is estimated that diabetes costs the U.S. economy US\$15 billion each year (Lurie 1988), while Parkinson's and Huntington's diseases, affecting more than 600 000 people in the United States, are estimated together to cost the U.S. economy over US\$3 billion annually (U.S. Congress 1990). Thus,

human fetal tissue transplantation therapies, if effective, may ultimately reduce overall health care costs, as well as improve patient health.

These issues should be resolved deliberately, not by default. Policy recommendations on the use of human fetal cadaver tissue may, to be effective, require force of law: the absence of specific legislation can lead to ambiguous and erratic directives (Delacourt 1988). Considering international experience, unenforced policy recommendations on fetal tissue use would not likely meet with uniform voluntary compliance. Embodiment of certain recommendations in guidelines of granting agencies or professional licensing bodies may suffice for particular aspects. As a starting point for deliberation, the following policies, incorporating proposals published elsewhere (Peel et al. 1972; National Health and Medical Research Council of Australia 1984; Council of Europe 1987; Mahowald et al. 1987b; Areen 1988; Calne and McGee 1988; Caplan 1988; Dickens 1988, 1989, 1993; U.S. National Institutes of Health 1988b; Annas and Elias 1989a; Greely et al. 1989; United Kingdom 1989; Lowy 1989; Freedman 1989; Marshall 1989; Barnes and Stevenson 1989; American Medical Association 1990; U.S. Congress 1990; Nelson 1990), could be recommended:

- Enforceable guidelines should be established for approval of human fetal cadaver tissue collection and distribution facilities and procedures. Comprehensive records of tissue collection, distribution, and use should be reported to a central registry.
- Research and clinical use of human fetal cadaver tissue should be permitted, encouraged, and supported by appropriate government funding bodies. However, such use should be permitted only in institutions with approved ethical review boards, and only on the basis of written protocols approved by the ethical review boards of all institutions involved. Protocol approval should require the demonstration that the particular tissue use is scientifically, medically, and ethically warranted; that superior alternatives to the use of human fetal cadaver tissue are unavailable; that where fetal tissue is derived from abortions, a clear separation is maintained between all aspects of the abortion procedure and the tissue use; that it is used only by investigators possessing all necessary skills, experience, and facilities; that experimental design meets rigorous scientific standards to ensure that results can be interpreted; and that fetal tissue is obtained only from approved sources.
- Voluntary written consent for the use of fetal tissue should be obtained from the woman undergoing abortion before any such use. Consent should be requested only after the woman has given consent for the abortion itself, and in a manner making clear that her decision with respect to tissue use will not affect the quality of her medical care. When fetal tissue is to be used

for human transplantation or for the generation of propagated cell lines, specific consent for such uses should be obtained. Users of the fetal tissue should have no role in obtaining the consent. When transplantation of fetal tissue is proposed, informed consent to the implantation of such tissue with reference to its mode of origin (e.g., from induced abortions) should be obtained from the prospective recipient.

- Abortion procedures should be dictated by considerations of safety for the pregnant woman. They should not be altered with respect to technique or timing for reasons pertaining to the collection or subsequent use of fetal tissue; in particular, abortion procedures intended to yield an intact living fetus should be prohibited. When, notwithstanding these considerations, an intact fetus is obtained, it should not be used for any purpose until its death is determined by a physician not involved in subsequent use of the fetal tissue. This determination should be on the basis of cessation of fetal heartbeat and spontaneous movement, or by other criteria yet to be established. Personnel participating in an abortion procedure should not in any way participate in the use of the fetal tissue thereby obtained. In addition, they should not benefit from such use through authorship of publications, grant support, or any other means.
- Criteria for determination of death at different stages of fetal development should be established, on the basis of expert medical, scientific, and legal recommendations, subject to periodic review on the basis of developments in those fields.
- The sale of human fetal cadaver tissue should be prohibited. Whether approved clinics and agencies providing such tissue for approved uses could be reimbursed for legitimate documented expenses should be considered.
- The donation of fetal tissue for specific purposes or to designated recipients should be prohibited by law.
- Reliable empirical data pertaining to such ethical and social issues as the impact of fetal cadaver tissue use on abortion incidence should be collected.
- Research aimed at the development of safe and effective alternatives to use of human fetal cadaver tissue should be encouraged and supported by appropriate government funding bodies.
- An appropriately constituted panel should from time to time review new research and legal developments regarding the use of fetal tissue and make policy recommendations to the MRC and other appropriate government agencies.

- For clarity, tissue derived from dead fetuses should be described as "fetal cadaver tissue."

Acknowledgments

I thank Dr. D. Rasmusson, Dr. C. Goodyer, Dr. P. Manga, and Dr. D. Vawter; B.M. Bardsley, R.N., and the International Institute for the Advancement of Medicine; and M. Bilsky and other staff of the Royal Commission on New Reproductive Technologies for their important contributions. The assistance of N. Lauronse was invaluable. I remain, however, wholly responsible for any errors of fact or interpretation, and for all opinions and recommendations expressed.

References

Aebischer, P., et al. 1991. "Transplantation of Microencapsulated Bovine Chromaffin Cells Reduces Lesion-Induced Rotational Asymmetry in Rats." *Brain Research* 560: 43-49.

Afanasyev, B.V., V.A. Shatrov, and N. Balayan. 1989. "The Effect of Human and Rat Fetal Bone on Hematopoiesis *In Vitro* and *In Vivo*." *Hamatologie und Bluttransfusion* 32: 183-87.

Allen, Y.S., and T.R. Kershaw. 1989. "A Morphological Study of the Neuroblastoma-Glioma Hybrid Cell Line, NG108-15, in Culture and After Grafting to the Adult Rat Brain." *Neuroscience Letters* 103: 247-52.

American Medical Association. Council on Scientific Affairs and Council on Ethical and Judicial Affairs. 1990. "Medical Applications of Fetal Tissue Transplantation." *JAMA* 263: 565-70.

Ammann, A.J., et al. 1975. "Thymus Transplantation in Patients with Thymic Hypoplasia and Abnormal Immunoglobulin Synthesis." *Transplantation* 20: 457-66.

Anderson, C.A. 1988. "Testimony." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

Annas, G.J., and S. Elias. 1989a. "The Politics of Transplantation of Human Fetal Tissue." *New England Journal of Medicine* 320: 1079-82.

—. 1989b. "The Ethics of Research Using Human Fetal Tissue." *New England Journal of Medicine* 321: 1609.

Areen, J.C. 1988. "Statement on Legal Regulation of Fetal Tissue Transplantation." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

Arendt, T., et al. 1983. "Loss of Neurons in the Nucleus Basalis of Meynert in Alzheimer's Disease, Paralysis Agitans and Korsakoff's Disease." *Acta Neuropathologica* 61: 101-108.

Aspenberg, P., and E. Andolf. 1989. "Bone Induction by Fetal and Adult Human Bone Matrix in Athymic Rats." *Acta Orthopaedica Scandinavica* 60: 195-99.

Auerbach, A.D., et al. 1990. "Prenatal Identification of Potential Donors for Umbilical Cord Blood Transplantation for Fanconi Anemia." *Transfusion* 30: 682-87.

Auerbach, R. 1988. "Qualities of Fetal Cells and Tissues." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

August, C.S., et al. 1968. "Implantation of a Foetal Thymus, Restoring Immunological Competence in a Patient with Thymic Aplasia (DiGeorge's Syndrome)." *Lancet* (7 December): 1210-11.

Backlund, E.O., et al. 1985. "Transplantation of Adrenal Medullary Tissue to Striatum in Parkinsonism: First Clinical Trials." *Journal of Neurosurgery* 62: 169-73.

Bailey, H., and K.D. Keele. 1935. "Addison's Disease Treated by Adrenal Grafting." *Proceedings of the Royal Society of Medicine* 29: 42.

Bakay, R.A.E. 1991. "What We Have Learned from Primate Research." In *Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences*, ed. O. Lindvall, A. Björklund, and H. Widner. Amsterdam: Elsevier Science.

Bakay, R.A.E., and C.J. Herring. 1989. "Central Nervous System Grafting in the Treatment of Parkinsonism." *Stereotactic and Functional Neurosurgery* 53: 1-20.

Ballow, M., and L.R. Hyman. 1977. "Combination Immunotherapy in Chronic Mucocutaneous Candidiasis: Synergism Between Transfer Factor and Fetal Thymus Tissue." *Clinical Immunology and Immunopathology* 8: 504-12.

Baranov, A., et al. 1989. "Bone Marrow Transplantation After the Chernobyl Nuclear Accident." *New England Journal of Medicine* 321: 205-12.

Barnes, D.W., and R.E. Stevenson. 1989. "Meeting Report: Human Fetal Tissue Transplantation Research Panel." *In Vitro Cellular and Developmental Biology* 25 (January): 6-8.

Barry, D.I., et al. 1987. "Grafted Noradrenergic Neurons Suppress Seizure Development in Kindling-Induced Epilepsy." *Proceedings of the National Academy of Sciences of the United States of America* 84: 8712-15.

—. 1989. "Grafts of Fetal Locus Coeruleus Neurons in Rat Amygdala-Piriform Cortex Suppress Seizure Development in Hippocampal Kindling." *Experimental Neurology* 106: 125-32.

Benikova, E.A., et al. 1987. "Experience with the Treatment of Children with Diabetes Mellitus Using Allo- and Xenografts of Cultures of Pancreatic Islet Cells." *Problemy Endokrinologii (Mosk)* 33 (2): 19-22.

Berry, D.L., et al. 1977. "Analysis of the Biotransformation of Benzo[a]pyrene in Human Fetal and Placental Tissues with High-Pressure Liquid Chromatography." *European Journal of Cancer* 13: 667-75.

Bethke, K.P., et al. 1988. "Cultured Human Fetal Pancreatic Tissue Reverses Experimentally Induced Diabetes in Nude Mice." *Current Surgery* 45 (March-April): 123-26.

Björklund, A., and U. Stenevi. 1979. "Reconstruction of the Nigrostriatal Dopamine Pathway by Intracerebral Nigral Transplants." *Brain Research* 177: 555-60.

Björklund, A., et al. 1981. "Functional Reactivation of the Deafferented Neostriatum by Nigral Transplants." *Nature* 289: 497-99.

—. 1987. "Mechanisms of Action of Intracerebral Neural Implants: Studies on Nigral and Striatal Grafts to the Lesioned Striatum." *Trends in Neurosciences* 10: 509-16.

Blakeslee, S. 1989. "In Careful Test, Parkinson's Patient Shows Gains After Fetal-Cell Implant." *New York Times*, 2 May, 18.

Bliss, M. 1982. *The Discovery of Insulin*. Chicago: University of Chicago Press.

Blyumkin, V.N., et al. 1988. ["Floating Cultures Obtained from Fetal Human Thyroid Gland."] *Biulleten Ekperimentalnoi Biologii i Meditsiny* (Mosk) 106: 600-602. (Published in *Bulletin of Experimental Biology and Medicine* 106 [1989]: 1616-18.)

Bodley, R.S., et al. 1961. "Hypoplastic Anaemia Treated by Transfusion of Foetal Haemopoietic Cells." *British Medical Journal* (25 November): 1385-88.

Bopp, J., and J.T. Burtchaell. 1988. "Human Fetal Tissue Transplantation Research Panel: Statement of Dissent." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 1. Bethesda: National Institutes of Health.

Bridge, J.B., et al. 1960. "Toxic Marrow Failure Treated by a Homograft of Foetal Haemopoietic Tissue." *Lancet* (19 March): 629-32.

Brody, E.B. 1990. "Women's Rights and the Medical Use of Fetal Tissue: An International Perspective." *Rhode Island Medical Journal* 73 (2): 53-58; discussion 59-67.

Brooks, J.R., and G. Gifford. 1959. "Pancreatic Homotransplantation." *Transplantation Bulletin* 6 (1): 100-103.

Brown, J., et al. 1974. "Control of Experimental Diabetes Mellitus in Rats by Transplantation of Fetal Pancreases." *Science* 184: 1377-79.

—. 1984. "Fetal Pancreas as a Donor Organ." *World Journal of Surgery* 8: 152-57.

Broxmeyer, H.E., et al. 1989. "Human Umbilical Cord Blood as a Potential Source of Transplantable Hematopoietic Stem/Progenitor Cells." *Proceedings of the National Academy of Sciences of the United States of America* 86: 3828-32.

—. 1990. "Human Umbilical Cord Blood: A Clinically Useful Source of Transplantable Hematopoietic Stem/Progenitor Cells." *International Journal of Cell Cloning* 8 (Suppl. 1): 76-89; discussion 89-91.

Brundin, P., and H. Sauer. 1991. "Grafting Human Fetal Brain Tissue: A Practical Guide." In *Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences*, ed. O. Lindvall, A. Björklund, and H. Widner. Amsterdam: Elsevier Science.

Brundin, P., et al. 1985. "Cyclosporin A Increases Survival of Cross-Species Intrastriatal Grafts of Embryonic Dopamine-Containing Cells." *Experimental Brain Research* 60: 204-208.

—. 1986. "Behavioural Effects of Human Fetal Dopamine Neurons Grafted in a Rat Model of Parkinson's Disease." *Experimental Brain Research* 65: 235-40.

Bryant, S.G., and C.S. Brown. 1986. "Current Concepts in Clinical Therapeutics: Major Affective Disorders, Part 1." *Clinical Pharmacy* 5: 304-18.

Buckley, R.H. 1988. "Fetal Thymus Transplantation for the Correction of Congenital Absence of the Thymus (DiGeorge's Syndrome)." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

Buckley, R.H., et al. 1976. "Correction of Severe Combined Immunodeficiency by Fetal Liver Cells." *New England Journal of Medicine* 294: 1076-81.

Burtchaell, J.T. 1988. "Case Study: University Policy on Experimental Use of Aborted Fetal Tissue." *IRB: A Review of Human Subjects Research* 10 (July-August): 7-11.

Calne, D.B., and P.L. McGeer. 1988. "Tissue Transplantation for Parkinson's Disease." *Canadian Journal of Neurological Sciences* 15: 364-65.

Canalis, P. 1887. "Contribution à l'étude du développement et de la pathologie des capsules surrénales." *Internationale Monatsschrift für Anatomie und Physiologie* 4: 312-34.

Cannon, M.J., et al. 1990. "Epstein-Barr Virus Induces Aggressive Lymphoproliferative Disorders of Human B Cell Origin in SCID/hu Chimeric Mice." *Journal of Clinical Investigation* 85: 1333-37.

Caplan, A.L. 1988. "Testimony." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

Cefalo, R.C., and H.T. Engelhardt, Jr. 1989. "The Use of Fetal and Anencephalic Tissue for Transplantation." *Journal of Medicine and Philosophy* 14: 25-43.

Childress, J.F. 1989. "Ethical Criteria for Procuring and Distributing Organs for Transplantation." *Journal of Health Politics, Policy and Law* 14: 87-113.

Chin, B.H., et al. 1979. "Metabolism of Carbaryl by Kidney, Liver, and Lung from Human Postembryonic Fetal Autopsy Tissue." *Clinical Toxicology* 14 (5): 489-98.

Clark, S.C., and R. Kamen. 1987. "The Human Hematopoietic Colony Stimulating Factors." *Science* 236: 1229-37.

Cleveland, W.W., et al. 1968. "Foetal Thymic Transplant in a Case of DiGeorge's Syndrome." *Lancet* (7 December): 1211-14.

Clough, C.G., et al. 1990. "Brain Implants in Man Do Not Break Down the Blood-Brain Barrier to Dopamine and Domperidone." *Brain Research* 536: 318-20.

—. 1991. "Intracerebral Grafting of Fetal Dorsal Pons in Genetically Epilepsy-Prone Rats: Effects on Audiogenic-Induced Seizures." *Experimental Neurology* 112: 195-99.

Cochrum, K.C., et al. 1989. "MHC Antigens Persist on Human Fetal Pancreatic Islet Cells Even After Culture and Transplantation into Nude Mice." *Transplantation Proceedings* 21: 2653-56.

Collerton, D. 1986. "Cholinergic Function and Intellectual Decline in Alzheimer's Disease." *Neuroscience* 19 (1): 1-28.

Colten, H.R. 1972. "Ontogeny of the Human Complement System: *In Vitro* Biosynthesis of Individual Complement Components by Fetal Tissues." *Journal of Clinical Investigation* 51: 725-30.

Council of Europe. Parliamentary Assembly. 1987. "Recommendation 1046 (1986) on the Use of Human Embryos and Fetuses for Diagnostic, Therapeutic, Scientific, Industrial and Commercial Purposes." *Human Reproduction* 2: 67-68.

Crombleholme, T.M., and M.R. Harrison. 1990. "Transplantation of Fetal Organs." In *The Unborn Patient: Prenatal Diagnosis and Treatment*. 2d ed., ed. M.R. Harrison, M.S. Golbus, and R.A. Filly. Philadelphia: W.B. Saunders.

Crombleholme, T.M., et al. 1990. "Transplantation of Fetal Cells." In *The Unborn Patient: Prenatal Diagnosis and Treatment*. 2d ed., ed. M.R. Harrison, M.S. Golbus, and R.A. Filly. Philadelphia: W.B. Saunders.

—. 1991. "Transplantation of Fetal Cells." *American Journal of Obstetrics and Gynecology* 164: 218-30.

Culliton, B.J. 1992. "Needed: Fetal Tissue Research." *Nature* 355: 295.

Danilovs, J., et al. 1982. "Expression of HLA-DR Antigens in Human Fetal Pancreas Tissue." *Diabetes* 31 (Suppl. 4): 23-29.

—. 1983. "HLA-DR and HLA-A, B, C Typing of Human Fetal Tissue." *Tissue Antigens* 21: 296-308.

Das, G.D., and J. Altman. 1971. "Transplanted Precursors of Nerve Cells: Their Fate in the Cerebellums of Young Rats." *Science* 173: 637-38.

David, S., and A.J. Aguayo. 1981. "Axonal Elongation into Peripheral Nervous System 'Bridges' After Central Nervous System Injury in Adult Rats." *Science* 214: 931-33.

Davies, P., and A.J. Maloney. 1976. "Selective Loss of Central Cholinergic Neurons in Alzheimer's Disease." *Lancet* (25 December): 1403.

Dawidson, I., et al. 1988. "Cryopreserved Human Fetal Pancreas: A Source of Insulin-Producing Tissue?" *Cryobiology* 25 (2): 83-93.

Deckel, A.W., et al. 1983. "Reversal of Long-Term Locomotor Abnormalities in the Kainic Acid Model of Huntington's Disease by Day 18 Fetal Striatal Implants." *European Journal of Pharmacology* 93: 287-88.

Delacourt, S. 1988. "Epp Backs Curb on Use of Fetal Cells." *Globe and Mail*, 16 July.

Dick, J.E. 1991. "Immune-Deficient Mice as Models of Normal and Leukemic Human Hematopoiesis." *Cancer Cells* 3: 39-48.

Dickens, B.M. 1988. "Foreign Regulations and Guidelines." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

- . 1989. "Fetal Tissue Transplantation." *Transplantation/Implantation Today* 6 (February): 33-41.
- . 1993. "Legal Issues in Embryo and Fetal Tissue Research and Therapy." In *Background and Current Practice of Fetal Tissue and Embryo Research in Canada*, vol. 15 of the research studies of the Royal Commission on New Reproductive Technologies. Ottawa: Minister of Supply and Services Canada.
- Dolin, R., et al. 1970. "Establishment of Human Fetal Intestinal Organ Cultures for Growth of Viruses." *Journal of Infectious Diseases* 122: 227-31.
- Donovan, P. 1990. "Funding Restrictions on Fetal Research: The Implications for Science and Health." *Family Planning Perspectives* 22: 224-31.
- Drugan, A., W.J. Evans, and M.I. Evans. 1989. "Fetal Organ and Xenograft Transplantation." *American Journal of Obstetrics and Gynecology* 160: 289-93.
- Ducat, L. 1988. "The National Disease Research Interchange." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.
- Duchosal, M.A., et al. 1992. "Immunization of hu-PBL-SCID Mice and the Rescue of Human Monoclonal Fab Fragments Through Combinatorial Libraries." *Nature* 355: 258-62.
- Dunn, E.H. 1916-17. "Primary and Secondary Findings in a Series of Attempts to Transplant Cerebral Cortex in the Albino Rat." *Journal of Comparative Neurology* 27: 565-82.
- Dunnett, S.B., and L.E. Annett. 1991. "Nigral Transplants in Primate Models of Parkinsonism." In *Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences*, ed. O. Lindvall, A. Björklund, and H. Widner. Amsterdam: Elsevier Science.
- Dunnett, S.B., D.C. Rogers, and S.J. Richards. 1989. "Nigrostriatal Reconstruction After 6-OHDA Lesions in Rats: Combination of Dopamine-Rich Nigral Grafts and Nigrostriatal 'Bridge' Grafts." *Experimental Brain Research* 75: 523-35.
- Dunnett, S.B., et al. 1982. "Septal Transplants Restore Maze Learning in Rats with Fornix-Fimbria Lesions." *Brain Research* 251: 335-48.
- Dymecki, J., et al. 1990. "Human Fetal Dopamine Cell Transplantation in Parkinson's Disease." Paper presented at the Eric K. Fernstrom Symposium, "Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences." Lund, Sweden, 20-22 June.
- Eckhoff, D.E., H.W. Sollinger, and D.A. Hullet. 1991. "Selective Enhancement of Beta Cell Activity by Preparation of Fetal Pancreatic Prolslets and Culture with Insulin Growth Factor 1." *Transplantation* 51: 1161-65.
- Elias, K.A., et al. 1990. "Development of Human Fetal Xenograft Transplants in Diabetic Nude Mice." *Transplantation Proceedings* 22: 806-807.
- "Embryos to Lipstick?" 1985. *New Scientist* (10 October): 21.
- Ernfors, P., et al. 1989. "A Cell Line Producing Recombinant Nerve Growth Factor Evokes Growth Responses in Intrinsic and Grafted Central Cholinergic Neurons." *Proceedings of the National Academy of Sciences of the United States of America* 86: 4756-60.

Faldino, G. 1924. "Sullo sviluppo dei tessuti embrionali omoplastici innestati nella camera anteriore dell'occhio del coniglio." *Archivio di Scienze Biologiche* 5: 328-46.

Fanciulli, S. 1978. "Prime osservazioni sulla funzionalita di neovagine da omoinnesto di tessuto fetale" ["Preliminary Observations on the Function of a Neovagina from a Fetal Tissue Homograft"]. *Mitnerva Ginecologica* 30 (1-2): 87-98.

Farkas, G.Y., G.Y. Lázár, and J. Herczegh. 1990. "The Long-Term Cultivation and Cryopreservation of Human Fetal Pancreatic Tissue." *Hormone and Metabolic Research* 25 (Suppl.): 64-68.

Fawcett, J.W. 1992. "Intrinsic Neuronal Determinants of Regeneration." *Trends in Neurosciences* 15 (1): 5-8.

Federlin, K., R.G. Bretzel, and B.J. Hering. 1991. "Recent Achievements in Experimental and Clinical Islet Transplantation." *Diabetic Medicine* 8 (1): 5-12.

Fichera, G. 1928. "Implanti omoplastici feto-umani nel cancro e nel diabete." *Tumori* 14: 434-77.

Fine, A. 1986. "Transplantation in the Central Nervous System." *Scientific American* 255 (August): 52-58B.

- . 1988. "The Ethics of Fetal Tissue Transplants." *Hastings Center Report* 18 (June-July): 5-8.
- . 1990. "Transplantation of Adrenal Tissue into the Central Nervous System." *Brain Research. Brain Research Reviews* 15: 121-33.

Fine, A., et al. 1985. "Cholinergic Ventral Forebrain Grafts into the Neocortex Improve Passive Avoidance Memory in a Rat Model of Alzheimer's Disease." *Proceedings of the National Academy of Sciences of the United States of America* 82: 5227-30.

- . 1988. "Transplantation of Embryonic Marmoset Dopaminergic Neurons to the Corpus Striatum of Marmosets Rendered Parkinsonian by 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine." *Progress in Brain Research* 78: 479-89.

Flake, A.W., et al. 1986. "Auxiliary Transplantation of the Fetal Liver. I. Development of a Sheep Model." *Journal of Pediatric Surgery* 21: 515-20.

Fleischman, R.A., and B. Mintz. 1979. "Prevention of Genetic Anemias in Mice by Microinjection of Normal Hematopoietic Stem Cells into the Fetal Placenta." *Proceedings of the National Academy of Sciences of the United States of America* 76: 5736-40.

Flicker, C., et al. 1983. "Behavioral and Neurochemical Effects Following Neurotoxic Lesions of a Major Cholinergic Input to the Cerebral Cortex in the Rat." *Pharmacology, Biochemistry and Behavior* 18: 973-81.

Formby, B., et al. 1988. "Growth Hormone Stimulates Insulin Gene Expression in Cultured Human Fetal Pancreatic Islets." *Journal of Clinical Endocrinology and Metabolism* 66: 1075-79.

Foster, G.A., et al. 1990. "Restoration of Function to the Denervated Spinal Cord After Implantation of Embryonic 5HT- and Substance P-Containing Raphe Neurones." *Progress in Brain Research* 82: 247-59.

Freed, C.R., et al. 1990. "Transplantation of Human Fetal Dopamine Cells for Parkinson's Disease: Results at 1 Year." *Archives of Neurology* 47: 505-12.

—. 1991. "Fetal Neural Implants for Parkinson's Disease: Results at 15 Months." In *Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences*, ed. O. Lindvall, A. Björklund, and H. Widner. Amsterdam: Elsevier Science.

Freed, W.J. 1983. "Functional Brain Tissue Transplantation: Reversal of Lesion-Induced Rotation by Intraventricular Substantia Nigra and Adrenal Medulla Grafts, with a Note on Intracranial Retinal Grafts." *Biological Psychiatry* 18: 1205-67.

—. 1990. "Fetal Brain Grafts and Parkinson's Disease." *Science* 250 (December): 1434.

Freed, W.J., U. Patel-Vaidya, and H.M. Geller. 1986. "Properties of PC12 Pheochromocytoma Cells Transplanted to the Adult Rat Brain." *Experimental Brain Research* 63: 557-66.

Freed, W.J., et al. 1981. "Transplanted Adrenal Chromaffin Cells in Rat Brain Reduce Lesion-Induced Rotational Behaviour." *Nature* 292: 351-52.

—. 1989. "Transplantation of B16/C3 Melanoma Cells into the Brains of Rats and Mice." *Brain Research* 485: 349-62.

Freedman, B. 1988. "The Ethics of Using Human Fetal Tissue." *IRB: A Review of Human Subjects Research* 10 (6): 1-4.

—. 1989. "Fetal Tissue Transplantation: Politics, Not Policy." *Canadian Medical Association Journal* 141: 1230-32.

Freeman, T.B., and J.H. Kordower. 1991. "Human Cadaver Embryonic Substantia Nigra Grafts: Effects of Ontogeny, Pre-Operative Graft Preparation and Tissue Storage." In *Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences*, ed. O. Lindvall, A. Björklund, and H. Widner. Amsterdam: Elsevier Science.

Gage, F.H., et al. 1985. "Anatomical and Functional Consequences of Grafting Mesencephalic Neurons into a Peripheral Nerve 'Bridge' Connected to the Denervated Striatum." *Experimental Brain Research* 60: 584-89.

Gale, K., and M.J. Iadarola. 1980. "Seizure Protection and Increased Nerve-Terminal GABA: Delayed Effects of GABA Transaminase Inhibition." *Science* 208: 288-91.

Gale, R.P. 1987a. "Development of the Immune System in Human Fetal Liver." *Thymus* 10: 45-56.

—. 1987b. "Fetal Liver Transplantation in Aplastic Anemia and Leukemia." *Thymus* 10: 89-94.

Gale, R.P., and Y. Reisner. 1988. "The Role of Bone-Marrow Transplants After Nuclear Accidents." *Lancet* (23 April): 923-26.

Garvey, J.F., P.J. Morris, and P.R. Millard. 1979. "Early Rejection of Allogeneic Foetal Rat Pancreas." *Transplantation* 27 (5)(May): 342-44.

Gash, D., J.R. Sladek, Jr., and C.D. Sladek. 1980. "Functional Development of Grafted Vasopressin Neurons." *Science* 210: 1367-69.

Gash, D.M. 1988. "Neural Implantation of Cultured Cells." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

Gash, D.M., et al. 1991. "Adrenal Medullary Transplants in Primates." In *Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences*, ed. O. Lindvall, A. Björklund, and H. Widner. Amsterdam: Elsevier Science.

Geyer, S.J., et al. 1985. "Immunogenetic Aspects of Transplantation in the Rat Brain." *Transplantation* 39: 244-47.

Gillam, L., ed. 1989. *The Fetus as Tissue Donor: Use or Abuse?* Conference proceedings. Melbourne: Monash University, Centre for Human Bioethics.

Githens, J.H., et al. 1973. "Grafting of Fetal Thymus and Hematopoietic Tissue in Infants with Immune Deficiency Syndromes." *Transplantation* 15: 427-34.

Gluckman, E., et al. 1989. "Hematopoietic Reconstitution in a Patient with Fanconi's Anemia by Means of Umbilical-Cord Blood from an HLA-Identical Sibling." *New England Journal of Medicine* 321: 1174-78.

Golbus, M.S., and D. Bauer. 1990. "Transplantation of Hematopoietic Stem Cells." In *The Unborn Patient: Prenatal Diagnosis and Treatment*. 2d ed., ed. M.R. Harrison, M.S. Golbus, and R.A. Filly. Philadelphia: W.B. Saunders.

Gold, R.B., and D. Lehrman. 1989. "Fetal Research Under Fire: The Influence of Abortion Politics." *Family Planning Perspectives* 21: 6-11.

Golde, D.W. 1991. "The Stem Cell." *Scientific American* 265 (December): 86-93.

Goldsobel, A.B., A. Haas, and E.R. Stiehm. 1987. "Bone Marrow Transplantation in DiGeorge's Syndrome." *Journal of Pediatrics* 111: 40-44.

Goodwin, W.E., et al. 1963. "Human Renal Transplantation, I. Clinical Experiences with Six Cases of Renal Homotransplantation." *Journal of Urology* 89: 13-24.

Greely, H.T., and T. Raffin. 1989. "The Ethics of Research Using Human Fetal Tissue." *New England Journal of Medicine* 321: 1610.

Greely, H.T., et al. 1989. "The Ethical Use of Human Fetal Tissue in Medicine." *New England Journal of Medicine* 320: 1093-96.

Groscurth, P., et al. 1986. "Cryopreservation of Human Fetal Organs." *Anatomy and Embryology* 174: 105-13.

Groth, C.G., et al. 1980. "Transplantation of Fetal Pancreatic Microfragments via the Portal Vein to a Diabetic Patient." *Diabetes* 29 (Suppl. 1): 80-83.

Gumpel, M., et al. 1983. "Survival and Differentiation of Oligodendrocytes from Neural Tissue Transplanted into New-Born Mouse Brain." *Neuroscience Letters* 37: 307-11.

—. 1987. "Transplantation of Human Embryonic Oligodendrocytes into Shiverer Brain." *Annals of the New York Academy of Sciences* 495: 71-85.

Gunning, J. 1990. *Human IVF, Embryo Research, Fetal Tissue for Research and Treatment, and Abortion: International Information*. London: Her Majesty's Stationery Office.

Gustavii, B. 1989. "Fetal Brain Transplantation for Parkinson's Disease: Technique for Obtaining Donor Tissue." *Lancet* (11 March): 565.

Haase, H. 1987. "Explanatory Memorandum to Council of Europe, Parliamentary Assembly Recommendation 1046 (1986)(1) on the Use of Human Embryos and Fetuses for Diagnostic, Therapeutic, Scientific, Industrial and Commercial Purposes." *Human Reproduction* 2: 68-75.

Hahn von Dorsche, H., and K. Falt. 1990. "Immunocytes in the Human Fetal Pancreas — A Contribution to Developmental Immunoendocrinology Concerning Diabetes Mellitus and Organ Cultivation." *Anatomischer Anzeiger* 171: 95-104.

Hahn von Dorsche, H., K. Falt, and H.J. Hahn. 1990. "Ontogenesis of Human Fetal Pancreas in Use for Transplantation." *Hormone and Metabolic Research* 25 (Suppl.): 227-33.

Hahn von Dorsche, H., H. Reiher, and H.J. Hahn. 1984. "Quantitative-Histologic Studies of Human Fetal Pancreas from Metabolically Healthy and Insulin-Dependent Diabetic Women." *Acta Anatomica* 118: 139-43.

Han, J.R., et al. 1990. ["Clinical and Experimental Study of Treating Aplastic Anemia with Fetal Liver Cell Suspension and Fetal Liver Cell-Free Suspension."] *Chung Hua Nei Ko Tsa Chih* [Chinese Journal of Internal Medicine] 29: 347-49, 382.

Hansen, J.T., and J.R. Sladek, Jr. 1989. "Fetal Research." *Science* 246: 775-79.

Hansmann, H. 1989. "The Economics and Ethics of Markets for Human Organs." *Journal of Health Politics, Policy and Law* 14: 57-85.

Hantraye, P., et al. 1990. "An Experimental Primate Model of Huntington's Disease: Behavioral and Anatomical Studies of Unilateral Excitotoxic Lesions of the Caudate-Putamen in the Baboon." *Experimental Neurology* 108: 91-104.

Harboe, M., et al. 1966. "Synthesis of Donor Type Gamma-G-Globulin Following Thymus Transplantation in Hypo-Gamma-Globulinaemia with Severe Lymphocytopenia." *Scandinavian Journal of Haematology* 3: 351-74.

Harousseau, J.L., et al. 1980. "Greffé de foie foetal dans les aplasies médullaires très sévères." *Nouvelle Revue française d'Hématologie* 22 (Suppl. 1): 50.

Hellerstrom, C., et al. 1988. "Experimental Pancreatic Transplantation in Diabetes." *Diabetes Care* 11 (Suppl. 1): 45-53.

—. 1989. "Aspects of Pancreatic Islet Transplantation in Diabetes Mellitus." *Baillière's Clinical Gastroenterology* 3: 851-63.

Highfield, R. 1988. "Transplant Success for Diabetics." *Daily Telegraph* (London), 20 August, 4.

Hisanaga, K., D.E. Bredesen, and F.E. Sharp. 1991. "A Central Neuronal-Like Cell Line Immortalized with a Retrovirus Encoding the Temperature-Sensitive SV40 Large T Antigen." *Society for Neuroscience - Abstracts* 17: 32.

Hitchcock, E.R., et al. 1988. "Transplantation in Parkinson's Disease: Stereotactic Implantation of Adrenal Medulla and Foetal Mesencephalon." *Acta Neurochirurgica Supplementum* 46: 48-50.

- . 1990a. "Stereotactic Implantation of Foetal Mesencephalon (STIM): The UK Experience." *Progress in Brain Research* 82: 723-28.
- . 1990b. "Stereotaxic Implantation of Foetal Mesencephalon." *Stereotactic and Functional Neurosurgery* 54-55: 282-89.
- . 1991. "Stereotactic Implantation of Foetal Mesencephalon." In *Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences*, ed. O. Lindvall, A. Björklund, and H. Widner. Amsterdam: Elsevier Science.

Hofman, F.M., J.A. Danilovs, and C.R. Taylor. 1984. "HLA-DR (Ia)-Positive Dendritic-Like Cells in Human Fetal Nonlymphoid Tissues." *Transplantation* 37: 590-94.

Houle, J.D., and P.J. Reier. 1989. "Regrowth of Calcitonin Gene-Related Peptide (CGRP) Immunoreactive Axons from the Chronically Injured Rat Spinal Cord into Fetal Spinal Cord Tissue Transplants." *Neuroscience Letters* 103: 253-58.

Huffaker, T.K., et al. 1989. "Xenografting of Fetal Pig Ventral Mesencephalon Corrects Motor Asymmetry in the Rat Model of Parkinson's Disease." *Experimental Brain Research* 77: 329-36.

Huisjes, H.J. 1984. *Spontaneous Abortion*. Edinburgh: Churchill Livingstone.

Hullett, D.A., et al. 1987. "Human Fetal Pancreas — A Potential Source for Transplantation." *Transplantation* 43: 18-22.

- . 1989. "Improved Human Fetal Pancreatic Tissue Survival Following Hyperbaric Oxygen Culture." *Transplantation Proceedings* 21: 2659-60.

Hurst, A.F., W.E. Tanner, and A.A. Osman. 1922. "Addison's Disease with Severe Anaemia Treated by Suprarenal Grafting." *Proceedings of the Royal Society of Medicine* 15: 19-20.

Itaka, K., et al. 1978. "Transplantation of Cadaver Kidneys from Anencephalic Donors." *Journal of Pediatrics* 93: 216-20.

"Immortal Cells: Grow Your Own Brain." 1990. *Economist* 314 (6 January): 85.

Isacson, O., S.B. Dunnett, and A. Björklund. 1986. "Graft-Induced Behavioral Recovery in an Animal Model of Huntington Disease." *Proceedings of the National Academy of Sciences of the United States of America* 83: 2728-32.

Isacson, O., et al. 1984. "Functional Neuronal Replacement by Grafted Striatal Neurones in the Ibotenic Acid-Lesioned Rat Striatum." *Nature* 311: 458-60.

Izzi, T., et al. 1985. "Fetal Liver Transplant in Aplastic Anemia and Acute Leukemia." *Progress in Clinical and Biological Research* 193: 237-49.

Jaeger, C.B. 1985. "Immunocytochemical Study of PC12 Cells Grafted to the Brain of Immature Rats." *Experimental Brain Research* 59: 615-24.

Johnson, M.P., et al. 1989. "Genetic Correction of Hereditary Disease." *Fetal Therapy* 4 (Suppl. 1): 28-39.

Jones, A.H., et al. 1977. "Bioactivation of Procarcinogens to Mutagens in Human Fetal and Placental Tissues." *Life Sciences* 21: 1831-35.

Jones, D.G. 1991. "Fetal Neural Transplantation: Placing the Ethical Debate Within the Context of Society's Use of Human Material." *Bioethics* 5: 23-43.

Jonsen, A.R. 1988. "Transplantation of Fetal Tissue: An Ethicist's Viewpoint." *Clinical Research* 36: 215-19.

Juchau, M.R., and M.J. Namkung. 1974. "Studies on the Biotransformation of Naphthalene-1,2-Oxide in Fetal and Placental Tissues of Humans and Monkeys." *Drug Metabolism and Disposition: The Biological Fate of Chemicals* 2: 380-85.

Juchau, M.R., M.G. Pederson, and K.G. Symms. 1972. "Hydroxylation of 3,4-Benzpyrene in Human Fetal Tissue Homogenates." *Biochemical Pharmacology* 21 (16)(15 August): 2269-72.

Juchau, M.R., et al. 1978. "Biotransformation and Bioactivation of 7,12-Dimethylbenz[a]anthracene in Human Fetal and Placental Tissues. Analyses of HPLC Profiles and Studies with *Salmonella typhimurium*." *Drug Metabolism and Disposition: The Biological Fate of Chemicals* 6: 273-81.

Kamo, H., et al. 1987. "Transplantation of Cultured Human Adrenal Chromaffin Cells into 6-Hydroxydopamine-Lesioned Rat Brain." *Synapse* 1: 324-28.

Kansal, V., et al. 1979. "Fetal Liver Transplantation in Aplastic Anemia." *Acta Haematologica* 62: 128-36.

Kantoff, P.W., et al. 1989. "In Utero Gene Transfer and Expression: A Sheep Transplantation Model." *Blood* 73: 1066-73.

Karpati, G., et al. 1989. "Dystrophin Is Expressed in mdx Skeletal Muscle Fibers After Normal Myoblast Implantation." *American Journal of Pathology* 135: 27-32.

Karson, E.M., and W.F. Anderson. 1990. "Prospects for Gene Therapy." In *The Unborn Patient: Prenatal Diagnosis and Treatment*. 2d ed., ed. M.R. Harrison, M.S. Golbus, and R.A. Filly. Philadelphia: W.B. Saunders.

Kawamoto, J.C., and J.N. Barrett. 1986. "Cryopreservation of Primary Neurons for Tissue Culture." *Brain Research* 384: 84-93.

Kearney, W., D.E. Vawter, and K.G. Gervais. 1991. "Fetal Tissue Research and the Misread Compromise." *Hastings Center Report* 21 (September-October): 7-12.

Keightley, R.G., et al. 1975. "Successful Fetal Liver Transplantation in a Child with Severe Combined Immunodeficiency." *Lancet* (1 November): 850-53.

Kelemen, E. 1973. "Recovery from Chronic Idiopathic Bone Marrow Aplasia of a Young Mother After Intravenous Injection of Unprocessed Cells from the Liver (and Yolk Sac) of Her 22mm CR-Length Embryo: A Preliminary Report." *Scandinavian Journal of Haematology* 10: 305-308.

Kemp, J.A., et al. 1978. "Reversal of Diabetes in Rats Using Fetal Pancreas Stored at -196° C." *Transplantation* 26: 260-64.

Kinnaert, P., et al. 1984. "Transplantation of Kidneys from Anencephalic Donors." *Transplantation Proceedings* 16: 71-72.

Kline, J., et al. 1992. "Fetal Tissue Supply." *Science* 257: 1189-90.

Kochupillai, V., et al. 1987a. "Fetal Liver Infusion in Aplastic Anemia." *Thymus* 10: 95-102.

—. 1987b. "Fetal Liver Infusion in Acute Myelogenous Leukaemia." *Thymus* 10: 117-24.

Kolarik, J., and P. Nadvornik. 1989. "Transplantation of Embryonic Brain Tissue (EBT) into the Schizophrenic Brain." In *9th International Congress of Neurological Surgery*, ed. R. Bhatia and S. Bhatia. 8-13 October, New Delhi, India.

Kolata, G. 1989. "More U.S. Curbs Urged in the Use of Fetal Tissue." *New York Times*, 19 November, 1.

Korsgren, O., et al. 1988. "Large-Scale Production of Fetal Porcine Pancreatic Isletlike Cell Clusters: An Experimental Tool for Studies of Islet Cell Differentiation and Xenotransplantation." *Transplantation* 45: 509-14.

Krestow, M., et al. 1991. "Xenotransplantation of Microencapsulated Fetal Rat Islets." *Transplantation* 51: 651-55.

Krieger, D.T., et al. 1982. "Brain Grafts Reverse Hypogonadism of Gonadotropin Releasing Hormone Deficiency." *Nature* 298: 468-71.

Krowka, J.F., et al. 1991. "Human T-Cells in the SCID-hu Mouse Are Phenotypically Normal and Functionally Competent." *Journal of Immunology* 146: 3751-56.

Kuhn, F., et al. 1990. "Tissue Bank of Human Fetal Pancreas." *Transplantation Proceedings* 22: 683-84.

Labbe, R., et al. 1983. "Fetal Brain Transplant: Reduction of Cognitive Deficits in Rats with Frontal Cortex Lesions." *Science* 221: 470-72.

Lacy, P.E., et al. 1991. "Maintenance of Normoglycemia in Diabetic Mice by Subcutaneous Xenografts of Encapsulated Islets." *Science* 254: 1782-84.

Lasnitzki, I. 1968. "The Effect of a Hydrocarbon-Enriched Fraction of Cigarette Smoke Condensate on Human Fetal Lung Grown *In Vitro*." *Cancer Research* 28: 510-13.

Lavrik, S.S., et al. 1990. ["Cryopreservation of a Suspension of Fetal Liver Cells for Clinical Use."] *Vrachebnœ Delo*: 90-93.

Law, P.K., et al. 1990. "Dystrophin Production Induced by Myoblast Transfer Therapy in Duchenne Muscular Dystrophy." *Lancet* (14 July): 114-15.

—. 1991. "Myoblast Transfer: The First Successful Cell Therapy in Treating Mammalian Genetic Diseases." *Society for Neuroscience - Abstracts* 17: 159.

Lawler, S.D. 1981. "Conception and Development of the Fetal Tissue Bank." *Journal of Clinical Pathology* 34: 240-48.

Le Gros Clark, W.E. 1940. "Neuronal Differentiation in Implanted Foetal Cortical Tissue." *Journal of Neurology and Psychiatry* 3: 263-84.

Lenschow D.J., et al. 1992. "Long-Term Survival of Xenogeneic Pancreatic Islet Grafts Induced by CTLA4lg." *Science* 257: 789-92.

Leonard, D.K., et al. 1989. "Prednisone, Azathioprine, and Cyclosporine A Toxicity on Human Fetal Pancreas." *Journal of Surgical Research* 46: 625-32.

Lewin, T. 1987. "Medical Use of Fetal Tissues Spurs New Abortion Debate." *New York Times*, 16 August, 1.

Li, L.X., and J.E. Turner. 1988. "Inherited Retinal Dystrophy in the RCS Rat: Prevention of Photoreceptor Degeneration by Pigment Epithelial Cell Transplantation." *Experimental Eye Research* 47: 911-17.

- . 1991. "Optimal Conditions for Long-Term Photoreceptor Cell Rescue in RCS Rats: The Necessity for Healthy RPE Transplants." *Experimental Eye Research* 52: 669-79.
- Lim, F., and A.M. Sun. 1980. "Microencapsulated Islets as Bioartificial Endocrine Pancreas." *Science* 210: 908-10.
- Lindvall, O. 1991a. "Intracerebral Transplantation of Fetal Dopamine Neurons in Sweden: Clinical Experiences from Four Patients with Idiopathic Parkinson's Disease." In *Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences*, ed. O. Lindvall, A. Björklund, and H. Widner. Amsterdam: Elsevier Science.
- . 1991b. "Prospects of Transplantation in Human Neurodegenerative Diseases." *Trends in Neurosciences* 14: 376-84.
- Lindvall, O., et al. 1988. "Fetal Dopamine-Rich Mesencephalic Grafts in Parkinson's Disease." *Lancet* (24 December): 1483-84.
- . 1989. "Human Fetal Dopamine Neurons Grafted into the Striatum in Two Patients with Severe Parkinson's Disease: A Detailed Account of Methodology and a 6-Month Follow-Up." *Archives of Neurology* 46: 615-31.
- . 1990. "Grafts of Fetal Dopamine Neurons Survive and Improve Motor Function in Parkinson's Disease." *Science* 247: 574-77.
- . 1992. "Transplantation of Fetal Dopamine Neurons in Parkinson's Disease: One-Year Clinical and Neurophysiological Observations in Two Patients with Putaminal Implants." *Annals of Neurology* 31: 155-65.
- Lissing, J.R., B.E. Tuch, and M.G. Suranyi. 1988. "The Use of Gliotoxin in Human Fetal Pancreas Transplantation." *Transplantation Proceedings* 20: 76-78.
- Lopez-Lozano, J.J., et al. 1991. "Can an Analogy Be Drawn Between the Clinical Evolution of Parkinson's Patients Who Undergo Autoimplantation of Adrenal Medulla and Those of Fetal Ventral Mesencephalon Transplant Recipients?" In *Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences*, ed. O. Lindvall, A. Björklund, and H. Widner. Amsterdam: Elsevier Science.
- Loudon, M.M., and E.N. Thompson. 1988. "Severe Combined Immunodeficiency Syndrome, Tissue Transplant, Leukaemia, and Q Fever." *Archives of Disease in Childhood* 63: 207-209.
- Lowy, F.H. 1989. "Fetal Tissue Transplantation: Time for a Canadian Policy." *Canadian Medical Association Journal* 141: 1227-29.
- Lucarelli, G., et al. 1980. "Fetal Liver Transplantation in Aplastic Anemia and Acute Leukemia." In *Fetal Liver Transplantation*, ed. G. Lucarelli, T.M. Fliedner, and R.P. Gale. Amsterdam: Excerpta Medica.
- Luisi, M. 1978. "Creazione di neovagina con innesto di tessuti fetal" [Creation of a Neovagina with a Fetal Tissue Graft]. *Minerva Ginecologica* 30: 299-316.
- Lurie, C. 1988. "Transplantation Research in Insulin-Dependent Diabetes." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

McCullagh, P. 1987. *The Fetus as Transplant Donor: Scientific, Social and Ethical Perspectives*. New York: John Wiley and Sons.

McCune, J.M., et al. 1988. "The SCID-hu Mouse: Murine Model for the Analysis of Human Hematolymphoid Differentiation and Function." *Science* 241: 1632-39.

—. 1990. "Suppression of HIV Infection in AZT-Treated SCID-hu Mice." *Science* 247: 564-66.

MacKenzie, D. 1985. "Third-World Kidneys for Sale." *New Scientist* 105 (28 March): 7.

Madrazo, I., et al. 1987. "Open Microsurgical Autograft of Adrenal Medulla to the Right Caudate Nucleus in Two Patients with Intractable Parkinson's Disease." *New England Journal of Medicine* 316: 831-34.

—. 1988. "Transplantation of Fetal Substantia Nigra and Adrenal Medulla to the Caudate Nucleus in Two Patients with Parkinson's Disease." *New England Journal of Medicine* 318: 51.

—. 1990a. "Fetal Homotransplants (Ventral Mesencephalon and Adrenal Tissue) to the Striatum of Parkinsonian Subjects." *Archives of Neurology* 47: 1281-85.

—. 1990b. "Neural Transplantation (Auto-Adrenal, Fetal Nigral and Fetal Adrenal) in Parkinson's Disease: The Mexican Experience." *Progress in Brain Research* 82: 593-602.

—. 1991a. "Fetal Ventral Mesencephalon Brain Homotransplantation in Parkinson's Disease: The Mexican Experience." In *Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences*, ed. O. Lindvall, A. Björklund, and H. Widner. Amsterdam: Elsevier Science.

—. 1991b. "Fetal Neural Grafting for the Treatment of Huntington's Disease (HD) — Report of the First Case." *Society for Neuroscience — Abstracts* 17: 902.

—. 1991c. "Development of Human Neural Transplantation." *Neurosurgery* 29: 165-77.

Mahowald, M.B., J. Silver, and R.A. Ratcheson. 1987a. "The Ethical Options in Transplanting Fetal Tissue." *Hastings Center Report* 17 (February): 9-15.

Mahowald, M.B., et al. 1987b. "Transplantation of Neural Tissue from Fetuses." *Science* 235: 1307-1308.

Mandel, T.E. 1984. "Transplantation of Organ-Cultured Fetal Pancreas: Experimental Studies and Potential Clinical Application in Diabetes Mellitus." *World Journal of Surgery* 8: 158-68.

Mann, D.M., and P.O. Yates. 1983. "Pathological Basis for Neurotransmitter Changes in Parkinson's Disease." *Neuropathology and Applied Neurobiology* 9: 3-19.

Markowski, B., and S.D. Lawler. 1977. "Use of Early Fetal Tissues Obtained from Suction Termination of Pregnancy." *Lancet* (22 January): 186-88.

Marsden, C.D. 1991. "Key Issues in Intracerebral Transplantation in Movement Disorders." In *Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences*, ed. O. Lindvall, A. Björklund, and H. Widner. Amsterdam: Elsevier Science.

Marshall, T.D. 1989. "Canadian Regulation of the Medical Use of Fetal Tissue in Research and Therapy." *Canadian Medical Association Journal* 140: 1021-22.

Mauerhoff, T., et al. 1988. "Differential Expression and Regulation of Major Histocompatibility Complex (MHC) Products in Neural and Glial Cells of the Human Fetal Brain." *Journal of Neuroimmunology* 18: 271-89.

Mazur, P., J.A. Kemp, and R.H. Miller. 1976. "Survival of Fetal Rat Pancreases Frozen to -78 and -196 Degrees." *Proceedings of the National Academy of Sciences of the United States of America* 73: 4105-4109.

Medical Research Council of Canada. 1991. *List of MRC Grants and Awards 1990-1991*. Ottawa: MRC Canada.

Metcalf, D., and M.A.S. Moore. 1971. "Embryonic Aspects of Haemopoiesis." In *Frontiers of Biology: Haemopoietic Cells*, vol. 24, ed. A. Neuberger and E.L. Tatum. Amsterdam: North Holland.

Miletitch, R.S., K.S. Bankiewicz, and R.J. Plunkett. 1990. "Fetal Brain Grafts and Parkinson's Disease." *Science* 250: 1434-35.

Miller, R.B. 1989. "On Transplanting Human Fetal Tissue: Presumptive Duties and the Task of Casuistry." *Journal of Medicine and Philosophy* 14: 617-40.

Mitus, A., et al. 1970. "Cultures of Segments of Human Fetal Intestine: Applications to Cytologic and Virologic Investigations." *Proceedings of the Society for Experimental Biology and Medicine* 134: 800-806.

Molina, H., et al. 1991. "Transplantation of Human Fetal Mesencephalic Tissue in Caudate Nucleus as Treatment for Parkinson's Disease: The Cuban Experience." In *Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences*, ed. O. Lindvall, A. Björklund, and H. Widner. Amsterdam: Elsevier Science.

Motojima, K., S. Matsuo, and Y. Mullen. 1989. "DR Antigen Expression on Vascular Endothelium and Duct Epithelium in Fresh or Cultured Human Fetal Pancreata in the Presence of Gamma-Interferon." *Transplantation* 48: 1022-26.

Mudrick, L.A., and K.G. Baimbridge. 1991. "Hippocampal Neurons Transplanted into Ischemically Lesioned Hippocampus: Anatomical Assessment of Survival, Maturation and Integration." *Experimental Brain Research* 86: 233-47.

Mudrick, L.A., K.G. Baimbridge, and M.J. Peet. 1989. "Hippocampal Neurons Transplanted into Ischemically Lesioned Hippocampus: Electroresponsiveness and Reestablishment of Circuitries." *Experimental Brain Research* 76: 333-42.

Mullen, Y.S., et al. 1977. "Complete Reversal of Experimental Diabetes Mellitus in Rats by a Single Fetal Pancreas." *Science* 195: 68-70.

Murray, C.L., and H.C. Fibiger. 1985. "Learning and Memory Deficits After Lesions of the Nucleus Basalis Magnocellularis: Reversal by Physostigmine." *Neuroscience* 14: 1025-32.

Nadaud, D., et al. 1984. "Functional Recovery Following Transplantation of Ventral Mesencephalic Cells in Rat Subjected to 6-OHDA Lesions of the Mesolimbic Dopaminergic Neurons." *Brain Research* 304: 137-41.

Nakahata, T., and M. Ogawa. 1982. "Hemopoietic Colony-Forming Cells in Umbilical Cord Blood with Extensive Capability to Generate Mono- and Multipotential Hemopoietic Progenitors." *Journal of Clinical Investigation* 70: 1324-28.

Namikawa, R., et al. 1988. "Infection of the SCID-hu Mouse by HIV-1." *Science* 242: 1684-86.

—. 1990. "Long-Term Hematopoiesis in the SCID-hu Mouse." *Journal of Experimental Medicine* 172: 1055-63.

Namkung, M.J., P.K. Zachariah, and M.R. Juchau. 1977. "O-Sulfonation of N-Hydroxy-2-Fluorenlyacetamide and 7-Hydroxy-N-2-Fluorenlyacetamide in Fetal and Placental Tissues of Humans and Guinea Pigs." *Drug Metabolism and Disposition: The Biological Fate of Chemicals* 5: 288-94.

National Health and Medical Research Council of Australia. 1984. "Ethics in Medical Research Involving the Human Fetus and Human Fetal Tissue." *Medical Journal of Australia* (12 May): 610-20.

Nelson, J.L. 1992. "Transplantation Through a Glass Darkly." *Hastings Center Report* 22 (September-October): 6-8.

Nelson, R.M. 1990. "A Policy Concerning the Therapeutic Use of Human Fetal Tissue in Transplantation." *Western Journal of Medicine* 152: 447-48.

Newmark, P. 1989. "Edging Towards Human Gene Therapy." *Nature* 342: 221.

Nilsson, O.G., P. Brundin, and A. Björklund. 1990. "Amelioration of Spatial Memory Impairment by Intrahippocampal Grafts of Mixed Septal and Raphe Tissue in Rats with Combined Cholinergic and Serotonergic Denervation of the Forebrain." *Brain Research* 515: 193-206.

Nolan, K. 1988. "Genug ist Genug: A Fetus Is Not a Kidney." *Hastings Center Report* 18 (December): 13-19.

Nothias, F., et al. 1990. "Double Step Neural Transplants to Replace Degenerated Motoneurons." *Progress in Brain Research* 82: 239-46.

Nozawa, M., et al. 1991. "Effects of Fetal Liver Transplantation in Rats with Congenital Metabolic Disease." *Transplantation Proceedings* 23: 889-91.

Ochiya, T., et al. 1989. "An In Vitro System for Infection with Hepatitis B Virus That Uses Primary Fetal Hepatocytes." *Proceedings of the National Academy of Sciences of the United States of America* 86: 1875-79.

Olson, L. 1988. "Parkinson's Disease Fetal Tissue Transplant Research, Basic and Clinical Studies." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

Olson, L., and A. Seiger. 1972. "Brain Tissue Transplanted to the Anterior Chamber of the Eye. 1. Fluorescence Histochemistry of Immature Catecholamine and 5-Hydroxytryptamine Neurons Reinnervating the Rat Iris." *Zeitschrift für Zellforschung und Mikroskopische Anatomie* 135: 175-94.

Olson, L., et al. 1987. "Human Fetal Tissues, Grafted to Rodent Hosts: Structural and Functional Observations of Brain, Adrenal and Heart Tissues *In Oculo" Experimental Brain Research* 67: 163-78.

O'Reilly, R.J., N. Kapoor, and D. Kirkpatrick. 1980. "Primary Immunodeficiencies." In *Primary Immunodeficiencies*, ed. M. Seligman and W.H. Hitzig. New York: Elsevier.

O'Reilly, R.J., et al. 1983. "Fetal Liver Transplantation in Man and Animals." In *Recent Advances in Bone Marrow Transplantation*, ed. R.F. Gale. New York: Alan R. Liss.

—. 1988. "Fetal Liver Transplantation in Man." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

Pacifci, G.M., and A. Rane. 1982. "Metabolism of Styrene Oxide in Different Human Fetal Tissues." *Drug Metabolism and Disposition: The Biological Fate of Chemicals* 10: 302-305.

Parodi, U. 1904. "Dell' innesto della capsula surrenale fetale." *Sperimentale* 58: 47-66.

Partridge, T.A., et al. 1989. "Conversion of mdx Myofibres from Dystrophin-Negative to -Positive by Injection of Normal Myoblasts." *Nature* 337: 176-79.

Peck, P. 1991. "Fetal Graft Stops Gargoylism." *Medical Tribune* 32 (12 December): 1, 8.

Peel, J., et al. 1972. *The Use of Fetuses and Fetal Material for Research*. Report of the Advisory Group, Department of Health and Social Security, Scottish Home and Health Department, Welsh Office. London: Her Majesty's Stationery Office.

Perlow, M.J., K. Kumakura, and A. Guidotti. 1980. "Prolonged Survival of Bovine Adrenal Chromaffin Cells in Rat Cerebral Ventricles." *Proceedings of the National Academy of Sciences of the United States of America* 77: 5278-81.

Perlow, M.J., et al. 1979. "Brain Grafts Reduce Motor Abnormalities Produced by Destruction of Nigrostriatal Dopamine System." *Science* 204: 643-47.

Perry, E.K., et al. 1985. "Cholinergic Correlates of Cognitive Impairment in Parkinson's Disease: Comparisons with Alzheimer's Disease." *Journal of Neurology, Neurosurgery and Psychiatry* 48: 413-21.

Peterson, C.M., et al. 1989. "Human Fetal Pancreas Transplants." *Journal of Diabetic Complications* 3: 27-34.

Phan, D.T., et al. 1989. "Human Fetal Liver as a Valuable Source of Haemopoietic Stem Cells for Allogeneic Bone Marrow Transplantation." *Haematologia* 22: 25-35.

Phillips, R.A., M.A.S. Jewett, and B.L. Gallie. 1989. "Growth of Human Tumors in Immune-Deficient Scid Mice and Nude Mice." *Current Topics in Microbiology and Immunology* 152: 259-63.

Phipps, P.H., et al. 1989. "Rapid Detection of Influenza Virus Infections in Human Fetal Lung Diploid Cell Cultures." *Journal of Infection* 18: 269-78.

Polezhaev, L.V., and M.A. Alexandrova. 1984. "Transplantation of Embryonic Brain Tissue into the Brain of Adult Rats After Hypoxic Hypoxia." *Journal für Hirnforschung* 25: 99-106.

Privat, A., H. Monsour, and M. Geffard. 1988. "Transplantation of Fetal Serotonin Neurons into the Transected Spinal Cord of Adult Rats: Morphological

Development and Functional Influence." *Progress in Brain Research* 78: 155-66.

Pynnonen, S., et al. 1977. "Carbamazepine: Placental Transport, Tissue Concentrations in Foetus and Newborn, and Level in Milk." *Acta Pharmacologica et Toxicologica* 41: 244-53.

Rafael, H., and V. Ayulo. 1991. "Infantile Cerebral Palsy: Actual Status and Future Treatment Possibilities." *Salud Publica Mexico* 33: 184-89.

Rauhala, A. 1988. "Slight Increase Noted in Hospital Abortions." *Globe and Mail*, 14 December.

Rayfield, L.S., et al. 1987. "Human Fetal Lymphocytes Require T-Cell Growth Factors for Cytotoxic Responses." *Clinical and Experimental Immunology* 69: 451-58.

Raymond, J.G. 1989. "The International Traffic in Women: Women Used in Systems of Surrogacy and Reproduction." *Reproductive and Genetic Engineering* 2: 51-57.

Reddy, S., and R.B. Elliott. 1988. "Ontogenetic Development of Peptide Hormones in the Mammalian Fetal Pancreas." *Experientia* 44: 1-9.

Redmond, D.E., Jr., et al. 1990. "Fetal Neural Graft Survival." *Lancet* (29 September): 820-22.

Rettie, A.E., et al. 1986. "Valproate Hydroxylation by Human Fetal Tissues and Embryotoxicity of Metabolites." *Clinical Pharmacology and Therapeutics* 40: 172-77.

Reynolds, B.A., and S. Weiss. 1992. "Generation of Neurons and Astrocytes from Isolated Cells of the Adult Mammalian Central Nervous System." *Science* 255: 1707-1709.

Richter-Levin, G., and M. Segal. 1989. "Raphe Cells Grafted into the Hippocampus Can Ameliorate Spatial Memory Deficits in Rats with Combined Serotonergic/Cholinergic Deficiencies." *Brain Research* 478: 184-86.

Ricordi C., et al. 1987. "Low-Temperature Culture of Human Islets or *In Vivo* Treatment with L3T4 Antibody Produces a Marked Prolongation of Islet Human-to-Mouse Xenograft Survival." *Proceedings of the National Academy of Sciences of the United States of America* 84: 8080-84.

Ridgeway, J. 1980. "Fetuses." In *Who Owns the Earth*. New York: Macmillan.

Ridley, R.M., et al. 1986. "Learning Impairment Following Lesion of the Basal Nucleus of Meynert in the Marmoset: Modification by Cholinergic Drugs." *Brain Research* 376: 108-16.

—. 1991. "Cholinergic Neural Transplants into Hippocampus Restore Learning Ability in Monkeys with Fornix Transections." *Experimental Brain Research* 83: 533-38.

Roberts, J.D. 1988. "The Intentional Creation of Fetal Tissue for Transplants: The Womb as a Fetus Farm?" *John Marshall Law Review* 21: 853-80.

Robertson, J.A. 1988a. "Rights, Symbolism, and Public Policy in Fetal Tissue Transplants." *Hastings Center Report* 18 (December): 5-12.

- . 1988b. "Fetal Tissue Transplants." *Washington University Law Quarterly* 66: 443-98.
- . 1990. "The Ethical Acceptability of Fetal Tissue Transplants." *Transplantation Proceedings* 22: 1025-27.
- Rojas, C.V., and E.P. Hoffman. 1991. "Recent Advances in Dystrophin Research." *Current Opinion in Neurobiology* 1: 420-29.
- Rosenberg, M.B., et al. 1988. "Grafting Genetically Modified Cells to the Damaged Brain: Restorative Effects of NGF Expression." *Science* 242: 1575-78.
- Rosenbluth, J., et al. 1990. "Myelin Formation Following Transplantation of Normal Fetal Glia into Myelin-Deficient Rat Spinal Cord." *Journal of Neurocytology* 19: 718-30.
- Rosner, F., et al. 1989. "Fetal Therapy and Surgery: Fetal Rights Versus Maternal Obligations." *New York State Journal of Medicine* 89 (February): 80-84.
- Rowley, P.T., B.M. Ohlsson-Wilhelm, and B.A. Farley. 1978. "Erythroid Colony Formation from Human Fetal Liver." *Proceedings of the National Academy of Sciences of the United States of America* 75: 984-88.
- Rozental, R.L., et al. 1988. ["Treatment of Labile Forms of Diabetes Mellitus by Transplantation of Cultured Pancreatic Islet Cells."] *Problemy Endokrinologii (Mosk)* 34 (1): 10-12.
- Sagen, J., and G.D. Pappas. 1987. "Morphological and Functional Correlates of Chromaffin Cell Transplants in CNS Pain Modulatory Regions." *Annals of the New York Academy of Sciences* 495: 306-33.
- Sagen, J., C.E. Sortwell, and G.D. Pappas. 1990. "Monoaminergic Neural Transplants Prevent Learned Helplessness in a Rat Depression Model." *Biological Psychiatry* 28: 1037-48.
- Sagen, J., et al. 1991. "Pain Reduction by Adrenal Medullary Transplants in the Spinal Subarachnoid Space of Terminal Cancer Patients." *Society for Neuroscience — Abstracts* 17: 235.
- Sandler, S., et al. 1985. "Tissue Culture of Human Fetal Pancreas: Development and Function of B-Cells *In Vitro* and Transplantation of Explants to Nude Mice." *Diabetes* 34: 1113-19.
- . 1987a. "Tissue Culture of Human Fetal Pancreas: Effects of Human Serum on Development and Endocrine Function of Islet-Like Cell Clusters." *Diabetes* 36: 1401-1407.
- . 1987b. "Tissue Culture of Human Fetal Pancreas: Growth Hormone Stimulates the Formation and Insulin Production of Islet-Like Cell Clusters." *Journal of Endocrinology and Metabolism* 65: 1154-58.
- . 1989. "Tissue Culture of Human Fetal Pancreas: Effects of Nicotinamide on Insulin Production and Formation of Islet-Like Cell Clusters." *Diabetes* 38 (Suppl. 1): 168-71.
- Sawle, G.V., et al. 1992. "Transplantation of Fetal Dopamine Neurons in Parkinson's Disease: PET [¹⁸F]6-L-Fluorodopa Studies in Two Patients with Putaminal Implants." *Annals of Neurology* 31: 166-73.
- Scott, R. 1981. *The Body as Property*. New York: Viking.

Segovia, J., R. Meloni, and K. Gale. 1989. "Effect of Dopaminergic Denervation and Transplant-Derived Reinnervation on a Marker of Striatal GABAergic Function." *Brain Research* 493: 185-89.

Segovia, J., et al. 1991. "Transplants of Fetal Substantia Nigra Regulate Glutamic Acid Decarboxylase Gene Expression in Host Striatal Neurons." *Brain Research. Molecular Brain Research* 10: 359-62.

Seiger, A., and L. Olson. 1977. "Quantitation of Fiber Growth in Transplanted Central Monoamine Neurons." *Cell Tissue Research* 179: 285-316.

Seller, M.J., and P.E. Polani. 1966. "Experimental Chimerism in a Genetic Defect in the House Mouse *Mus musculus*." *Nature* 212 (1 October): 80-81.

Sever, J.L. 1980. "Infectious Causes of Human Reproductive Loss." In *Human Embryonic and Fetal Death*, ed. I.H. Porter and B. Hook. Toronto: Academic Press.

Sheedlo, H.J., L. Li, and J.E. Turner. 1991a. "Photoreceptor Cell Rescue at Early and Late RPE-Cell Transplantation Periods During Retinal Disease in RCS Dystrophic Rats." *Journal of Neural Transplantation and Plasticity* 2: 55-63.

Sheedlo, H.J., et al. 1991b. "Transplantation to the Diseased and Damaged Retina." *Trends in Neuroscience* 14: 347-50.

Shiogama, T., et al. 1990. "Cryopreservation of Human Fetal Pancreatic Tissues, Growth and Functional Maturation of Tissues Grafted into Athymic Mice." *Hormone and Metabolic Research (Suppl.)* 25: 239-41.

Shumakov, V.I., et al. 1980. ["Xenotransplantation of Cultures of Islet Cells of Fetal Human Pancreas into Experimentally Diabetic Rats."] *Bulleten Ekspertimentalnoi Biologii i Meditsiny (Mosk)* 89: 48-50.

Sieradzan, K., and G. Vrbova. 1991. "Factors Influencing Survival of Transplanted Embryonic Motoneurones in the Spinal Cord of Adult Rats." *Experimental Neurology* 114: 286-99.

Simeonovic, C.J., et al. 1980. "Modulation of Tissue Immunogenicity by Organ Culture: Comparison of Adult Islets and Fetal Pancreas." *Transplantation* 30: 174.

Simonova, L.I., et al. 1976. ["Processing and Transplantation of Preserved (-196 Degrees) Hemopoietic Fetal Tissue."] *Problemy Germatologii i Perelvaniia* 21: 20-23.

Simpson, A.M., B.E. Tuch, and P.C. Vincent. 1991. "Characterization of Endocrine-Rich Monolayers of Human Fetal Pancreas That Display Reduced Immunogenicity." *Diabetes* 40: 800-808.

Sladek, J.R., Jr., et al. 1987. "Transplantation of Fetal Dopamine Neurons into Primate Brain Reverses MPTP-Induced Parkinsonism." *Progress in Brain Research* 71: 309-23.

Sotelo, C., and R.M. Alvarado-Mallart. 1991. "The Reconstruction of Cerebellar Circuits." *Trends in Neurosciences* 14: 350-55.

Stefan, Y., et al. 1983. "A Quantitative Immunofluorescent Study of the Endocrine Cell Populations in the Developing Human Pancreas." *Diabetes* 32: 293-301.

Stenchever, M.A., J.M. Hempel, and M.N. Macintyre. 1965. "Maintenance of *In Vitro* Growth Ability and Chromosome Integrity Following the Deep Freezing of Minced Fetal Tissue." *Cryobiology* 1: 240-41.

Stevens, J.R., et al. 1988. "Cerebral Transplants for Seizures: Preliminary Results." *Epilepsia* 29: 731-37.

Stone, H.B., J.C. Owings, and G.O. Gey. 1938. "Further Reports on Grafting of Endocrine Glands." *Mississippi Doctor* 15 (March): 6-9.

Stromberg, I., et al. 1985. "Chronic Implants of Chromaffin Tissue into the Dopamine-Denervated Striatum. Effects of NGF on Graft Survival, Fiber Growth and Rotational Behavior." *Experimental Brain Research* 60: 335-49.

—. 1986. "Human Fetal Substantia Nigra Grafted to the Dopamine-Denervated Striatum of Immunosuppressed Rats: Evidence for Functional Reinnervation." *Neuroscience Letters* 71: 271-76.

—. 1989. "Human Fetal Mesencephalic Tissue Grafted to Dopamine-Denervated Striatum of Athymic Rats: Light- and Electron-Microscopical Histochemistry and *In Vivo* Chronoamperometric Studies." *Journal of Neuroscience* 9: 614-24.

Subrt, O., M. Tichy, and V. Vlayka. 1990. "Grafting of Fetal Dopamine Neurons in Parkinson's Disease." Paper presented at the Eric K. Fernstrom Symposium, "Intracerebral Transplantation in Movement Disorders: Experimental Basis and Clinical Experiences." Lund, Sweden, 20-22 June.

Suskova, V.S., et al. 1988. ["Changes in the C-Peptide Concentration in the Blood of Patients with Diabetes Mellitus After the Transplantation of Cultures of Pancreatic Islet Cells."] *Problemy Endokrinologii (Mosk)* 34 (4): 16-20.

Syvalahti, E. 1987. "Monoaminergic Mechanisms in Affective Disorders." *Medical Biology* 65: 89-96.

Taylor, R.M., et al. 1986. "Enzyme Replacement in Nervous Tissue After Allogeneic Bone-Marrow Transplantation for Fucosidosis in Dogs." *Lancet* (4 October): 772-74.

Tessler, A. 1991. "Intraspinal Transplants." *Annals of Neurology* 29: 115-23.

Thomas, E.D., et al. 1957. "Intravenous Infusion of Bone Marrow in Patients Receiving Radiation and Chemotherapy." *New England Journal of Medicine* 254: 491-96.

Thompson, L. 1992a. "Fetal Transplants Show Promise." *Science* 257: 868, 870.

—. 1992b. "Cell-Transplant Results Under Fire." *Science* 257: 472-74.

Thompson, W.G. 1890. "Successful Brain Grafting." *New York Medical Journal* 51: 701-702.

Thorne, E.D., and M. Michejda. 1989. "Fetal Tissue from Spontaneous Abortions: A New Alternative for Transplantation Research?" *Fetal Therapy* 4 (1): 37-42.

Touraine, J.-L. 1983. "Bone-Marrow and Fetal-Liver Transplantation in Immunodeficiencies and Inborn Errors of Metabolism: Lack of Significant Restriction of T-Cell Function in Long-Term Chimeras Despite HLA-Mismatch." *Immunological Reviews* 71: 103-21.

- . 1989. "New Strategies in the Treatment of Immunological and Other Inherited Diseases: Allogeneic Stem Cells Transplantation." *Bone Marrow Transplantation* 4 (Suppl. 4): 139-41.
- Touraine, J.-L., et al. 1987. "Fetal Tissue Transplantation, Bone-Marrow Transplantation and Prospective Gene Therapy in Severe Immunodeficiencies and Enzyme Deficiencies." *Thymus* 10: 75-87.
- . 1989. "In-Utero Transplantation of Stem Cells in a Patient with the Bare Lymphocyte Syndrome." *Lancet* (17 June): 1382.
- . 1991. "New Developments in Stem Cell Transplantation with Special Reference to the First *In Utero* Transplants in Humans." *Bone Marrow Transplantation* 7 (Suppl. 3): 92-97.
- Trucco, T. 1989. "Sales of Kidneys Prompt New Laws and Debate." *New York Times*, 1 August, C1.
- Tuch, B. 1988. "Immunogenicity of Fetal Tissue." *Hastings Center Report* 18 (August-September): 44.
- . 1991. "Reversal of Diabetes by Human Fetal Pancreas: Optimization of Requirements in the Hyperglycemic Nude Mouse." *Transplantation* 51: 557-62.
- Tuch, B.E., and K.A. Lenord. 1989. "Insulin Is Advantageous to the Growth of Human Fetal Pancreas After Its Implantation." *Transplantation Proceedings* 21: 3803-3804.
- Tuch, B.E., and R.S. Monk. 1991. "Regulation of Blood Glucose to Human Levels by Human Fetal Pancreatic Xenografts." *Transplantation* 51: 1156-60.
- Tuch, B.E., and K.J. Osgerby. 1990. "Maturation of Insulinogenic Response to Glucose in Human Fetal Pancreas with Retinoid Acid." *Hormone and Metabolic Research* 25 (Suppl.): 233-38.
- Tuch, B.E., S.M. Dunn, and D.V. de Vahl. 1990. "The Effect on Researchers of Handling Human Fetal Tissue." *Transplantation Proceedings* 22: 2109-10.
- Tuch, B.E., R.S. Monk, and J. Beretov. 1991. "Reversal of Diabetes in Athymic Rats by Transplantation of Human Fetal Pancreas." *Transplantation* 52: 172-74.
- Tuch, B.E., K.J. Osgerby, and J.R. Turtle. 1988. "Normalization of Blood Glucose Levels in Nondiabetic Nude Mice by Human Fetal Pancreas After Induction of Diabetes." *Transplantation* 46: 608-11.
- Tuch, B.E., et al. 1984a. "Transplantation of Human Fetal Pancreatic Tissue into Diabetic Nude Mice." *Transplantation Proceedings* 16: 1059-61.
- . 1984b. "Histologic Differentiation of Human Fetal Pancreatic Explants Transplanted into Nude Mice." *Diabetes* 33: 1180-87.
- . 1985. "Typing of Human Fetal Organs for the Histocompatibility Antigens A, B and DR." *Pathology* 17: 57-61.
- Tuddenham, E.G.D., et al. 1974. "Tissue Localization and Synthesis of Factor-VIII-Related Antigen in the Human Foetus." *British Journal of Haematology* 26: 669-77.
- Tulandi, T. 1991. "McGill Researchers Have Treated Ectopic Pregnancy Nonsurgically for Two Years." *Medical Post* 27 (5 March): 15.

Tulipan, N.B., H.A. Zatur, and G.S. Allen. 1985. "Pituitary Transplantation: Part 1. Successful Reconstitution of Pituitary-Dependent Hormone Levels." *Neurosurgery* 16: 331-35.

United Kingdom. Committee to Review the Guidance on the Research Uses of Fetuses and Fetal Material. 1989. *Report*. London: Her Majesty's Stationery Office.

United States. Congress. Office of Technology Assessment. 1990. *Neural Grafting: Repairing the Brain and Spinal Cord*. Washington, DC: U.S. Government Printing Office.

United States. National Institutes of Health. 1988a. *Human Fetal Tissue Transplantation Research. Report of the Advisory Committee to the Director, National Institutes of Health*. Bethesda: National Institutes of Health.

United States. National Institutes of Health. Human Fetal Tissue Transplantation Research Panel. 1988b. *Report of the Human Fetal Tissue Transplantation Research Panel*. 2 vols. Bethesda: National Institutes of Health.

Uphoff, D.E. 1958. "Preclusion of Secondary Phase of Irradiation Syndrome by Innoculation of Fetal Hematopoietic Tissue Following Lethal Total-Body X Irradiation." *Journal of the National Cancer Institute* 20: 625-32.

Usadel, K.H., et al. 1980. "Transplantation of Human Fetal Pancreas: Experience in Thymusaplastic Mice and Rats and in a Diabetic Patient." *Diabetes* 29 (Suppl. 1): 74-79.

Vandekerckhove, B.A.E., et al. 1991. "Clonal Analysis of the Peripheral T-Cell Compartment of the SCID-hu Mouse." *Journal of Immunology* 146: 4173-79.

Vawter, D.E., et al. 1990. *The Use of Human Fetal Tissue: Scientific, Ethical, and Policy Concerns*. Minneapolis: University of Minnesota, Center for Biomedical Ethics.

Verrier, E.D., et al. 1989. "Neonatal Model of Heterotopic Heart Transplantation in Pigs." *Journal of Thoracic and Cardiovascular Surgery* 98: 127-36.

Voss, H.F. 1988. "Testimony." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

Vossen, J.M. 1988. "Bone Marrow Transplantation in the Treatment of Severe Immunodeficiencies: Possibilities and Problems." *Immunological Investigations* 17: 135-46.

Walters, J.M. 1991. "Report from North America: Anencephalic Infants as Organ Sources." *Bioethics* 5: 326-41.

Warburton, D., et al. 1980. "Chromosome Abnormalities in Spontaneous Abortions: Data from the New York City Study." In *Human Embryonic and Fetal Death*, ed. I.H. Porter and E.B. Hook. Toronto: Academic Press.

Warren, M.A. 1978. "Can the Fetus Be an Organ Farm?" *Hastings Center Report* 8 (October): 23-25.

Wictorin, K., et al. 1990. "Reformation of Long Axon Pathways in Adult Rat Central Nervous System by Human Forebrain Neuroblasts." *Nature* 347: 556-58.

Winn, S.R., et al. 1991. "Behavioral Recovery Following Intrastriatal Implantation of Microencapsulated PC12 Cells." *Experimental Neurology* 113: 322-29.

Winter, H.S., et al. 1991. "Human Intestine Matures as Nude Mouse Xenograft." *Gastroenterology* 100: 89-98.

Wolff, J.A., et al. 1989. "Grafting Fibroblasts Genetically Modified to Produce L-Dopa in a Rat Model of Parkinson Disease." *Proceedings of the National Academy of Sciences of the United States of America* 86: 9011-14.

Wolohan, M.J., and D.J. Zaleske. 1991. "Hemiepiphysal Reconstruction Using Tissue Donated from Fetal Limbs in a Murine Model." *Journal of Orthopaedic Research* 9: 180-85.

Wong, G.H.W., et al. 1984. "Inducible Expression of H-2 and Ia Antigens on Brain Cells." *Nature* 310: 688-91.

—. 1985. "Interferon-Gamma Induces the Expression of H-2 and Ia Antigens on Brain Cells." *Journal of Immunology* 7: 255-78.

Wong, L. 1988. "Medical Research Council Tissue Bank." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

Woodruff, M.F.A. 1953. "Endocrine Glands." *Transplantation Bulletin* 1 (1): 8.

—. 1960. *The Transplantation of Tissues and Organs*. Springfield: C.C. Thomas.

Wu, Z.G., et al. 1989. "In Vitro Culture and Transplantation of Encapsulated Human Fetal Islets as an Artificial Endocrine Pancreas." *ASAIO Transactions* 35: 736-38.

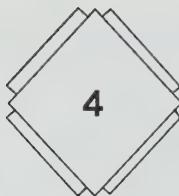
Yan, Z.B., et al. 1990. "A Study of Cadaveric Fetal Adrenal Used for Adrenal Transplantation to Treat Addison's Disease: Thirteen Cases Reported." *Transplantation Proceedings* 22: 280-82.

Yong, V.W., et al. 1989. "Transplantation of Human Sympathetic Neurons and Adrenal Chromaffin Cells into Parkinsonian Monkeys: No Reversal of Clinical Symptoms." *Journal of the Neurological Sciences* 94: 51-67.

Zaner, R.M. 1989. "Anencephalics as Organ Donors." *Journal of Medicine and Philosophy* 14: 61-78.

Zhu, R.P., X.Y. Lin, and J.T. Wang. 1990. ["Fetal Hepatocellular Suspension Transfusion (FHST) in the Treatment of Chronic Active Hepatitis B."] *Chung-Hua Nei Ko Tsa Chih* [Chinese Journal of Internal Medicine] 29: 419-21, 445.

Zimmermann, T., et al. 1979. "Oxidation and Synthesis of Fatty Acids in Human and Rat Placental and Fetal Tissues." *Biology of the Neonate* 36: 109-12.



Report on a Survey of Use and Handling of Human Reproductive Tissues in Canadian Health Care Facilities

SPR Associates Inc.



Executive Summary

In the period November 1991 to February 1992, a survey of Canadian health care facilities, which included all hospitals offering obstetric and gynaecological services and any other facilities performing pregnancy terminations, was conducted for the Royal Commission on New Reproductive Technologies.

The purpose of the survey was to identify how reproductive tissues obtained in these facilities were used or handled. Reproductive tissues were defined to include ova, ovarian tissue, embryos, abortuses/fetal tissue, and placentas. The survey resulted in an overall response rate of over 93% from those health care facilities offering such services.

This report presents an overview of the survey results. A number of findings of interest emerged, among them:

- There is a lack of awareness of reproductive technology issues among health care organizations and a lack of a commonly familiar terminology to discuss these issues.
- Established procedures for the handling and use of human reproductive tissues were generally unwritten in the majority of health care facilities surveyed. Overall, 28.6% of health

care facilities dealing with ovarian tissue reported written protocols, 31.9% of health care facilities dealing with abortuses reported written protocols for dealing with abortuses, and 26.4% of those dealing with placentas reported written protocols for dealing with placentas.¹

- While written protocols were somewhat more likely to be found in health care facilities in large urban areas, a general lack of written protocols was seen among organizations of all rural and urban size groups.
- Few consent forms were noted in the documentation provided by those health care facilities reporting that they did have written protocols.
- Few facilities retain tissues for in-house research: 0.8% of hospital/clinic facilities surveyed use ovarian tissue for research; 0.8% use abortuses/fetal tissue; 1.2% use placental tissue; 0.2% use ova; and 0.2% use embryos.
- Various tissues were sent to several types of organizations, with distribution (other than for disposal) to other institutions, researchers, or agencies.
- There is need for additional information to ascertain the end use of human reproductive tissues in a number of cases.² The following figures represent the percentages of health care facilities responding that they provide human reproductive tissues to other agencies, researchers, or institutions. The figures for each category were: 6.2% ovarian tissue; 12.9% abortuses/fetal tissue; and 9.5% placental tissue. No ova and no embryos were reported to be provided to other institutions, researchers, or agencies, so this issue does not arise for those categories of human tissue.
- The actual end use made of human reproductive tissues sent to recipients was not known to many of the administrators completing the survey. While known uses ranged from disposal (and burial) to laboratory and research activities to commercial uses, some administrators indicated that they simply did not know what was being done with these materials (it is probable that in a large hospital someone other than the person completing the questionnaire would know about the specific use). The researchers noted that some of the questions regarding end use could be clarified through a follow-up survey of the organizations identified as recipients of human reproductive tissues.

Introduction

Survey Objectives

In the period from November 1991 to February 1992, SPR Associates conducted a national survey on the handling and use of human reproductive tissues for the Royal Commission on New Reproductive Technologies. Human reproductive tissues were defined to include ova, ovarian tissue, embryos, abortuses/fetal tissue, and placentas.

The survey examined two types of health care facilities — the first group surveyed included all known public and private hospitals and health care facilities that offer obstetric and gynaecological services (739 facilities originally listed). These institutions (and the appropriate contact people within them) were identified by the Commission (fertility clinics as such were not surveyed, but hospitals in which fertility clinics were located were included in the survey). The areas to be examined in the hospital survey were as follows:

- any use of ova, embryos, ovarian tissue, fetal tissue/abortuses, or placentas for research projects within the institution;
- any transfer of ova, embryos, ovarian tissue, fetal tissue/abortuses, or placentas to other institutions or researchers either in Canada or elsewhere;
- any importation of ova, embryos, ovarian tissue, fetal tissue/abortuses, or placentas for use at the institution;
- any regulations, protocols, and guidelines governing research, including patient consent, handling, storage, disposal, and tracking; and
- sources and amounts of funding related to ova, embryos, ovarian tissue, fetal tissue, or placental research, handling, and transfer.

The second group surveyed were Canadian abortion clinics. These 17 facilities (and the contact people within them) were identified by the Commission. The areas examined in the abortion clinic survey paralleled those of the first component of the survey and were as follows:

- any use of fetal tissue/abortuses for research within the clinic;
- any transfer of fetal tissue/abortuses to other institutions or researchers in Canada or elsewhere;
- any importation of fetal tissue/abortuses to Canada for use in the clinics;
- any regulations, protocols, and guidelines governing research, including patient consent, handling, storage, and disposal; and
- sources and amounts of funding related to fetal tissue research, handling, and transfer.

Purpose of This Report

The purpose of SPR's work was to collect hospital and clinic data on the topics listed above in the survey objectives. Detailed analysis of protocols and consent forms was beyond the scope of this study and this information was forwarded to the Commission. The primary purpose of this report is to provide an overview of the survey's results, its successes and limitations, and the statistical patterns derived from its data.

In this way, by sharing what we have learned (including survey operations/insights), the report is seen as providing data previously unavailable in this country on the use and handling of human reproductive tissues.

The Survey

Questionnaires

The survey instruments, as originally drafted by the Commission, were pretested by SPR Associates. Two questionnaires were further developed: a longer questionnaire for hospitals and community clinics offering obstetric and gynaecological services and a comparable (but shorter) questionnaire for abortion clinics. These questionnaires are provided in Appendix 1 of this report.

Survey Procedures

The survey plan had several key steps in contacting the health organizations surveyed:

- provision of the initial survey mailing by courier to all urban-based organizations and by Canada Post Special Letter to hospitals and clinics in rural and remote locations;
- maintenance of a bilingual hotline to allow those who received the survey to telephone the survey office and ask questions, seek clarification, etc.;
- a variety of follow-ups by telephone, mail, fax, and courier to check on the status of the survey, to remind non-respondents to complete it, to locate questionnaires that had gone astray, or to identify cases where the questionnaire had been destroyed or lost (requiring another mailing); and
- where requested, completion of some surveys by telephone (generally done only with small rural hospitals).

As discussed in the section on data quality and limitations, the surveys were completed by a wide range of staff in the various organizations. In large urban hospitals, the survey may have been completed by an

administrator, a vice-president of medical services, a head of obstetric or gynaecological services, a director of nursing, or a director of a fertility clinic.

Response Rates

As a result of these procedures and contacts, an extremely good response rate was achieved overall — over 93% for abortion clinics and hospitals, and clinics with obstetric and gynaecological services. When abortion clinics were considered alone, a response rate of 76% was observed. This response rate was similar for most provinces and urban size groups (see Tables 1 and 2), but some special subgroups (abortion clinics and hospitals with fertility clinics) had somewhat lower response rates, probably because they started the survey slightly later than the other organizations surveyed (most health care facilities first received the survey in late November 1991, but health care facilities in Quebec and New Brunswick received the survey in the second week of December 1991, as did abortion clinics and hospitals with fertility clinics).

Table 1. Health Care Facility Survey Responses by Province/Territory

Province/territory	Number not returned	Number returned	Total number	% not returned*	% returned*
Alberta	9	102	111	8.1	91.9
British Columbia	2	86	88	2.3	97.7
Manitoba	4	63	67	6.0	94.0
New Brunswick	1	22	23	4.3	95.7
Newfoundland	5	25	30	16.7	83.3
Northwest Territories	0	5	5	0.0	100.0
Nova Scotia	0	34	34	0.0	100.0
Ontario	11	162	173	6.4	93.6
Prince Edward Island	1	4	5	20.0	80.0
Quebec	12	104	116	10.3	89.7
Saskatchewan	4	98	102	4.0	96.0
Yukon Territory	0	2	2	0.0	100.0
Totals**	49	707	756	6.5	93.5

* Percentages do not include an adjustment for non-sample cases that should not have been included in the survey to begin with (because they offered no obstetric/gynaecological services). A number of these counted above as responses (52) were found during the survey to have been included incorrectly in the original sample listing hospitals with obstetric/gynaecological services. It is not possible to adjust the response rate without knowing the proportion of non-sample cases among those who did not return the survey at all.

** Totals include questionnaires sent to both hospitals and abortion clinics.

Table 2. Health Care Facility Survey Responses by Size of Community

Type of area	Number not returned	Number returned	Total sample	% not returned	% returned
Rural areas	9	388	397	2.3	97.7
Small urban areas	24	183	207	11.6	88.4
Large urban areas	16	136	152	10.6	89.4
Totals	49	707	756	8.2	91.8

Type of area is a good indication of facility size, because all major hospitals, including university hospitals, are found in large urban areas. The table figures do not include an adjustment for non-sample cases that offer no obstetric/gynaecological services. Without also knowing non-sample non-return rate, we could not adjust the data.

Following completion of the survey, it was determined that 52 responding hospitals did not meet the original assumptions for inclusion in the survey sample — because they offered no obstetric/gynaecological services. These cases were deleted from subsequent analyses for this report.

Data Quality and Limitations

Generally, the quality of the information provided is rich and informative. However, to clarify some aspects, further follow-up would be needed. For example, many hospitals and clinics that reported the existence of written procedures have attached relatively thin sets of materials. There is no way, short of a follow-up survey, to determine if they sent everything. This may be a particular concern for consent forms, relatively few of which were attached to the returned questionnaires.

Similarly, inaccurate answers were sometimes provided because of the role of the person completing the questionnaire. For example, the survey for one major hospital known to house a fertility clinic was completed by the head of obstetric and gynaecological services and indicated "no fertility treatments." We presume this was because this person did not perceive the fertility clinic as part of the obstetric and gynaecological services, perhaps because it may be on an indirect administrative line of control in the hospital (seen as attached to the hospital but not part of it, or certainly not part of the obstetric/gynaecological unit). Mistakes in reporting occurred for several hospitals.

Overall, as is often the case with surveys, the process of doing the survey provided lessons as important as the answers to the survey itself.

Of these lessons, the most outstanding was probably the disinclination of many health organizations surveyed (particularly small rural hospitals) to imagine that new reproductive technologies had anything to do with them at all.

Indeed, a number of these smaller organizations threw the survey out upon receipt, had to be contacted by telephone, and then were sent another copy of the questionnaire. The significant lesson here is the extent to which the Commission faced obstacles in exploring these issues with the health system. Obstacles are also established by a lack of commonly accepted terminology. Terminology was a problem in several ways, for example, confusion between "research" uses of human tissues and "pathology," or confusion as to what constitutes an "abortion" (the survey did not include a technical definition of these terms). We believe some hospitals responded "no abortions" because they failed to include spontaneous abortions in their understanding of "abortion."

Uses and Distribution of Human Reproductive Tissues in Canadian Health Care Facilities with Obstetric and Gynaecological Services

Uses and Distribution of Ova

Only 6.1% of the health care facilities in the survey reported that they provided fertility treatments,³ and of these a majority reported that they only use fertility-enhancing drugs (no direct handling of ova or embryos). Therefore, those health care facilities reporting the use of only fertility-enhancing drugs had no further information for the detailed questions regarding handling of ova.

Only very small percentages of all health care facilities reported handling ova (1.2% of all health care facilities surveyed, or eight facilities; see Table 3). Of all the facilities surveyed, 0.8% (five facilities) handled ova and disposed of them in-house; 0.3% (two facilities) retained ova for use in other patients' fertility treatment; and 0.2% (one facility) retained ova for research. Of the eight health care facilities dealing with ova, four reported written protocols for the handling of ova. For these facilities, the median year of establishment of these protocols was 1989. Only one of the eight facilities reported plans for changing protocols in the next six months.

Table 3. Uses and Distribution of Ova Among All Health Care Facilities Surveyed

%*	Number of health care facilities**	Use/distribution
93.9	603	Not applicable, no fertility treatments***
4.8	31	Not applicable, no ova obtained in treatment (fertility treatments with drugs only)
0.8	5	Disposed of in-house
0.0	0	Disposed of through an outside agency, institution, or company
0.0	0	Retained, at least in part, for use in the patient's future fertility treatment
0.3	2	Retained, at least in part, for use in the fertility treatment of others at this facility
0.2	1	Retained, at least in part, for use in research in this facility
0.0	0	Provided, at least in part, to other institutions, agencies, or individual researchers

* Percentages are not intended to add to 100%. Percentages are based on the portion of all health care facilities from a total of 642 survey responses.

** The total of 642 includes the total number of questionnaires returned, minus 52 that were found to be included incorrectly in the original sample listing and minus the 13 abortion clinics that are calculated separately later in this report.

*** Eighteen facilities did not provide an answer as to whether they provided fertility treatments. In this table they have been combined with the "no fertility treatment" group.

Uses and Distribution of Embryos

As with handling and distribution of ova, only a very small proportion of all health care facilities surveyed (1.2%, or eight facilities) reported fertility treatments that could involve embryos (see Table 4). Among all the health care facilities surveyed, 0.9% (six facilities) handled embryos and disposed of them in-house; 0.6% (four facilities) retained embryos for use in the patient's future fertility treatment; 0.3% (two facilities) retained embryos for use in other patients' fertility treatment; and 0.2% (one facility) retained embryos for research.

Seven of the eight health care facilities dealing with embryos reported written protocols for handling of embryos. Among these, the median year for establishment of protocols was 1988. Only one facility reported anticipated changes in protocols in the coming six months.

Table 4. Uses and Distribution of Embryos Among All Health Care Facilities Surveyed

%*	Number of health care facilities	Use/distribution
93.9	603	Not applicable, no fertility treatments
4.8	31	Not applicable, no embryos obtained in treatment (fertility treatments with drugs only)
0.9	6	Disposed of in-house
0.0	0	Disposed of through an outside agency, institution, or company
0.6	4	Retained, at least in part, for use in the patient's future fertility treatment
0.3	2	Retained, at least in part, for use in the fertility treatment of others at this facility
0.2	1	Retained, at least in part, for use in research in this facility
0.0	0	Provided, at least in part, to other institutions, agencies, or individual researchers

* Percentages are not intended to add to 100%. Percentages are based on the portion of all health care facilities from a total of 642 survey responses. (Eight facilities did not provide an answer as to whether they provided fertility treatments. In this table they have been combined with the "no fertility treatment" group.)

Uses and Distribution of Ovarian Tissue

A large percentage of health care facilities reported that no ovarian tissue was handled at all (46.9%; see Table 5). Only an extremely small proportion of all facilities reported that ovarian tissue was provided to other agencies (6.2%), or that the material was retained for in-house research (0.8%).

Overall, hospitals and their clinics indicated few written protocols for handling of ovarian tissue. Only 28.6% of hospitals dealing with ovarian tissue (i.e., 96 facilities) reported that written protocols existed for use and distribution of such tissue. Of these, the median year of establishment was reported as 1981, with only a very small minority of facilities dealing with ovarian tissue (2.1%) reporting that changes were expected in the next six months. These results show that a minority have formal written guidelines, and these have a slow rate of change or modification.

Table 5. Uses and Distribution of Ovarian Tissue Among All Health Care Facilities Surveyed

%*	Number of health care facilities	Use/distribution
46.9	301	Not applicable, no ovarian tissue
27.9	179	Disposed of through an outside agency, institution, or company
21.3	137	Disposed of in-house
6.2	40	Provided, at least in part, to other institutions, agencies, or individual researchers
0.8	5	Retained, at least in part, for use in research in this facility

* Percentages are not intended to add to 100%. Percentages are based on the portion of all health care facilities from a total of 642 survey responses. (Five facilities provided no data on ovarian tissue.)

Uses and Distribution of Abortuses and Fetal Tissue

Many hospitals/clinics reported no abortuses (12.5%) and most others (86.9%) reported disposal (including burial) as the major form of distribution: 53.3% disposed of through outside agencies and 33.6% through in-house disposal (see Table 6). Some organizations reported that they supply fetal tissue to others (12.9%) or use such tissue for research of their own (0.8%).

Table 6. Uses and Distribution of Abortuses and Fetal Tissue Among All Health Care Facilities Surveyed

%*	Number of health care facilities	Use/distribution
12.5	80	Not applicable, no abortuses/fetal tissue
53.3	342	Disposed of through an outside agency, institution, or company
33.6	216	Disposed of in-house
12.9	83	Provided, at least in part, to other institutions, agencies, or individual researchers
0.8	5	Retained, at least in part, for use in research in this facility

* Percentages are not intended to add to 100%. Percentages are based on the portion of health care facilities providing valid responses from a total of 642 survey responses. (Eight facilities provided no data on abortuses.)

Most hospitals or clinics dealing with abortuses/fetal tissue indicated that they had no written protocols for handling such material, with 31.9% of these hospitals/clinics dealing with abortuses/fetal tissue (i.e., 177 facilities) reporting that written protocols existed. Of these, the median year of establishment was reported as 1981, with a minority of facilities dealing with abortuses/fetal tissue (6.1%) reporting that changes were expected in the next six months. As was previously noted regarding ovarian tissue, these results for abortuses/fetal tissue show a general absence of written guidelines and a slow rate of change in written guidelines.

Uses and Distribution of Placentas

Placentas were primarily disposed of in-house (66.7%), but 37.1% of responding organizations reported outside disposal (see Table 7). Again, only a few of all the facilities (9.5%) reported provision of placentas to other agencies or researchers, and only a small fraction (1.2%) reported keeping placentas for research uses.

Table 7. Uses and Distribution of Placentas Among All Health Care Facilities Surveyed

%*	Number of health care facilities	Use/distribution
2.8	18	Not applicable, no placentas
37.1	238**	Disposed of through an outside agency, institution, or company
66.7	428	Disposed of in-house
9.5	61**	Provided, at least in part, to other institutions, agencies, or individual researchers
1.2	8	Retained, at least in part, for use in research in this facility

* Percentages are not intended to add to 100%. Percentages are based on the portion of all health care facilities from a total of 642 survey responses. (Two facilities provided no data on placentas.)

** As detailed later in the follow-up survey data, in some cases placentas were mistakenly reported as disposed of, when, in fact, the recipient received the placentas for other uses.

Approximately 26.4% of the hospitals handling placentas (i.e., 164 facilities) reported that written protocols existed for the handling of placentas. Of these, the median year of establishment of the protocol was reported as 1983, with only a small minority of facilities handling placentas (8.4%) reporting that changes were expected in the next six months. Again,

these results show a general absence of formal written guidelines and a relatively slow rate of change or modification.

Provincial and Urban Size Patterns

Generally, only one provincial pattern emerges from this analysis of the survey data base — the higher-than-average incidence of written protocols in Ontario hospitals as compared to other provinces, and the lower-than-average incidence of written protocols in Quebec hospitals.

Written protocols are somewhat more likely to be found for hospitals in large urban centres, but this is only a moderate tendency. In fact, it appears that the majority of health care facilities in rural and small urban areas lack written protocols of any type, as do half or more of all health care facilities in large urban centres. While larger hospitals are more likely to have written protocols, many of the largest hospitals operate with no written guidelines on these matters.

Research Involving Human Reproductive Tissues

Generally, the survey results suggest that research on human reproductive tissues is to be found only in a very few hospitals — for the most part a subset of the largest ones. Altogether, only 1.8% (12) of the health care facilities reported such research projects. Of the 14 research projects reported, 10 projects had a total funding value of over \$1 million. The 14 topics being studied are listed below:

- chromosomal abnormalities in human oocytes;
- comprehensive assessment of sperm function in male infertility, using a novel diagnostic protocol;
- a case-control study of the placentas of stillborn and live-born infants for infectious agents;
- fetal tissue transplant for Parkinson's disease;
- prednisone/acetysalicylic acid (ASA) treatment in women with recurrent fetal loss;
- *in vitro* whole organ perfusion system;
- early preimplantation cell screening program;
- role of placental corticotropin-releasing factor;
- infection and premature labour;
- chromosome study of unfertilized ova and untransferred embryos;
- role of growth factors and angiogenesis in placenta;
- role of growth factors in development of human follicle;
- endocrine, receptor, and growth factor control in normal and polycystic ovarian tissue; and
- study of myometrial cells *in vitro*.

Distribution of Human Reproductive Tissues

Distribution

The pattern of distribution for reproductive tissues is shown in Table 8. As indicated in this table, the primary reason for distribution was for disposal purposes. However, we also found that a proportion of some human reproductive tissues (namely, 12.9% fetal tissue; 9.5% placental tissue; and 6.2% ovarian tissue) was made available for reasons other than disposal to other institutions, researchers, and agencies. No ova or embryos were made available to other institutions, researchers, or agencies.

Looking at specific patterns of distribution, slightly more than one thousand tissue distribution records were included in the final survey data base. A tissue distribution record involved one recipient identified by one health care facility as the recipient for one type of tissue. Many of these records were duplicates (e.g., 15 hospitals could identify one medical laboratory as a recipient for various tissues), so distillation of these results would identify about one hundred unique recipient organizations (funeral homes excluded).

Table 8. Distribution Patterns for Human Reproductive Tissues Among All Health Care Facilities Distributing Human Reproductive Tissues

%*	Number of health care facilities	Type of use
61.5	635	Disposal
24.6	254	Some other use (verbal explanations added)**
17.1	177	Treatment
16.7	173	Unsure of use
11.0	114	Research uses***

* Percentages are not intended to add to 100%. Percentages are based on responses from 642 health care facilities providing valid responses, and 1 033 distribution records.

** Variously comprising distributions for pathology, burial, and other purposes.

*** In many cases, the recipient (pathology laboratory) shows it is pathological examination that is done, not a research project per se.

Lack of Clarity Regarding End Uses

A potentially important feature is that the actual use made of outgoing human reproductive tissues by these recipients was not known to many of the administrators completing the survey. While known uses ranged from disposal (including burial) to specific laboratory and research activities to

commercial uses, some administrators (16.7% of distribution reports) indicated that they did not know specifically what was being done with these materials by the recipients. In large hospitals, someone else in the hospital might know about a specific use (see earlier discussion of data quality and limitations). It is possible that recipients may be using the materials for a purpose the hospital administrator is unaware of. It was hoped that such issues would be clarified through a follow-up survey of recipients of distributed human reproductive tissues.

Uses and Distribution of Human Reproductive Tissues Among Canadian Abortion Clinics

The 13 abortion clinics responding to the survey reported patterns in handling and use of abortuses that were generally very similar to those found for other health care facilities. Most clinics reported use of outside agencies for disposal (nine abortion clinics), with another two clinics reporting in-house disposal. There were no reported research uses in-house.

Overall, clinics sent abortuses mainly to hospitals for purposes of disposal (eight clinics). Two reported distribution for research purposes, one for treatment purposes, and three clinics reported distribution for other uses.

Like hospitals and other health clinics, abortion clinics were also unlikely to have written protocols for handling of abortuses. These were reported by only 15% (two) of the responding abortion clinics.

Conclusions

The survey has illustrated a number of important features about the use of and recipients of human reproductive tissues. Some of these features are listed below:

- There is a lack of awareness of reproductive technology issues and terminology among health care organizations.
- There is a general lack of formal (written) protocols and guidelines on handling of human reproductive tissues.
- Few hospitals and fertility clinics retained tissues for in-house research. The figures for each tissue category were as follows: 1.2% of hospitals/clinics (eight facilities) reported they retained placental tissue; 0.8% (five facilities) retained ovarian tissue; 0.8% (five facilities) retained abortuses or fetal tissue; 0.2% (one facility) retained ova; and 0.2% (one facility) retained embryos.

- There is a need to clarify actual use made of human reproductive tissues by recipients in a proportion of cases. Where the facility stated it forwarded such tissue to another institution, agency, or researchers, the proportion needing clarification was 19% for abortuses or fetal tissue, 17.6% for placental tissue, and 10.3% for ovarian tissue. No clarification was needed for either ova or embryos — no facilities reported providing these materials to other institutions, agencies, or individuals. The researchers noted that the verification of end uses by those receiving them could be undertaken through a survey of the recipient organizations, after analysis has generated a listing of them.

Appendix 1. Survey Questionnaires

Survey of Health Care Facilities Regarding Research Uses and Handling of Human Tissues

INTRODUCTION AND INSTRUCTIONS: The Royal Commission on New Reproductive Technologies needs information regarding a variety of topics including research projects recently completed, now in progress, or being actively considered in Canadian health care facilities, which involve the use of: (a) human fetal tissues (whole abortuses, their tissues and/or placentas); and (b) human ova, embryos or ovarian tissue (as the latter may be used for ovum maturation).

In addition, we are interested in information regarding the handling of these tissues other than for research (including sources of and disposal of tissues). This means that many of the questions will apply to your health care facility even if there is no research underway.

This information is needed from every hospital or facility in Canada that provides gynecological/obstetric services at any level, regardless of whether or not new reproductive technologies are in use in the facility and regardless of whether or not related research is conducted.

Please be assured that the information you provide will remain *strictly confidential*. Only aggregated data will be published. Where institutions, agencies, or researchers are identified by you as sources and/or recipients of tissues, and if they have not been contacted through the initial survey, we intend to forward a similar survey to them.

Most of the following questions require only a check mark or a brief written answer. In some places, when you choose an answer, you are asked to skip to another question further on. Please follow these instructions. Where text answers are requested, please type or complete using ink. In

some cases, we ask you to attach copies of documents (e.g. protocols/consent forms). Please be sure to attach these documents even though you may have provided them already to the Commission in connection with other projects/surveys. Be sure to read each question carefully to determine if it applies to your health care facility. *The questionnaire should be completed by the person in your organization most knowledgeable about these procedures.* Please follow the attached "instructions for return", to send the survey to: Survey of Health Care Facilities, Royal Commission on New Reproductive Technologies, 2318 Danforth Avenue, 2nd Floor, Toronto, Ontario, M4C 1K7. If you have any questions, please telephone the survey "hotline" at (416) 467-8430.

Thank you in advance for your time and assistance.

A. Research Projects/Activities

1. Do any research projects (completed in the past 12 months, now underway, or planned for in the next 12 months) in this health care facility use human embryos, ova, ovarian tissue, abortuses/fetal tissues, or placentas for purposes of research in any form?

No ----> **GO TO QUESTION #2(a), SECTION B**
 Yes ----> Please complete the attached yellow Research Project Form, then continue with **Question 2(a), Section B**

B. Fertility Treatment

- 2.(a) Are fertility treatments, such as IVF, ovulation enhancing drugs, etc. offered in this health care facility?

Yes ----> **GO TO QUESTION #2(b)**
 No ----> **GO TO QUESTION #8(a), SECTION C**
- 2.(b) In this facility, is there a written protocol for the handling of ova used in fertility treatment (including their disposal and/or storage)?

Yes ----> Please attach a copy of the written protocol and blank versions of all consent forms used (including blank versions of any consent forms signed by the woman herself)
 No ----> Please outline your procedure, including disposal and/or storage of ova:

2.(c) How long has this protocol/procedure been in use?
Since: _____ MONTH/YEAR)

2.(d) Do you anticipate any changes to this protocol/procedure in the near future, say, the next 6 months?

No
 Yes ----> Please elaborate:

3.(a) What happens to ova from a patient, which are not used for her fertility treatment in this health care facility? (CHECK AS MANY AS APPLY)

Disposed of in-house
 Disposed of through an outside agency, institution or company
 Retained, at least in part, for use in the patient's future fertility treatment
 Retained, at least in part, for use in fertility treatment of others at this facility
 Retained, at least in part, for use in research at this facility
 Provided, at least in part, to other institutions, agencies, companies or individual researchers

3.(b) For each outside recipient, specify:

Name of Outside Recipient*	Location (City/ Province)	Name of Contact Person	Phone Number	USE:			
				Treatment	Research	Disposal	Unsure
#1				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#2				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#3				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#4				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#5				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

(Attach additional lists if necessary)

* Outside recipients include health care institutions, individual researchers, agencies, disposal companies, funeral homes and/or related organizations, cemeteries, etc.

4.(a) Do you have a written protocol for the handling of ova not used in fertility treatment (including their disposal and/or storage)?

- Yes, included in protocol on handling of ova used during fertility treatment
- Yes, separate ----> Please attach a copy of the written protocol and blank versions of all consent forms used (including blank versions of any consent forms signed by the woman herself)
- No ----> Please outline your procedure, including disposal and/or storage of ova:

4.(b) How long has this protocol/procedure been in use?

Since: _____ (MONTH/YEAR)

4.(c) Do you anticipate any changes to this protocol/procedure in the near future, say, the next 6 months?

- No
- Yes ----> Please elaborate:

5.(a) Do you have a written protocol for the handling of embryos used in fertility treatment (including their disposal and/or storage)?

- Yes ----> Please attach a copy of the written protocol and blank versions of all consent forms used (including blank versions of any consent forms signed by the woman herself)
- No ----> Please outline your procedure, including disposal and/or storage of embryos:

5.(b) How long has this protocol/procedure been in use?
 Since: _____ (MONTH/YEAR)

5.(c) Do you anticipate any changes to this protocol/procedure in the near future, say, the next 6 months?

No
 Yes ----> Please elaborate:

6.(a) What happens to embryos not used in fertility treatment in this health care facility? (CHECK AS MANY AS APPLY)

Disposed of in-house
 Disposed of through an outside agency, institution or company
 Retained, at least in part, for use in the patient's future fertility treatment
 Retained, at least in part, for use in fertility treatment of others at this facility
 Retained, at least in part, for use in research at this facility
 Provided, at least in part, to other institutions, agencies, companies or individual researchers

6.(b) For each outside recipient, specify:

Name of Outside Recipient*	Location (City/ Province)	Name of Contact Person	Phone Number	USE:			
				Treatment	Research	Disposal	Unsure
#1				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#2				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#3				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#4				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#5				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

(Attach additional lists if necessary)

* Outside recipients include health care institutions, individual researchers, agencies, disposal companies, funeral homes and/or related organizations, cemeteries, etc.

7.(a) Do you have a written protocol for the handling of embryos not used in fertility treatment (including their disposal and/or storage)?

- Yes, included in protocol on handling of embryos during fertility treatment
- Yes, separate ----> Please attach a copy of the written protocol and blank versions of all consent forms used (including blank versions of any consent forms signed by the woman herself)
- No ----> Please outline your procedure, including disposal and/or storage of embryos:

7.(b) How long has this protocol/procedure been in use?

Since: _____ (MONTH/YEAR)

7.(c) Do you anticipate any changes to this protocol/procedure in the near future, say, the next 6 months?

- No
- Yes ----> Please elaborate:

C. Ovarian Tissue

8.(a) What happens to ovarian tissue in this health care facility? (CHECK AS MANY AS APPLY)

- Not applicable/no ovarian tissue ----> **GO TO QUESTION #10(a), NEXT PAGE**
- Disposed of in-house
- Disposed of through an outside agency, institution or company
- Retained, at least in part, for use in research at this facility
- Provided, at least in part, to other institutions, agencies or individual researchers

8.(b) For each outside recipient, specify:

Name of Outside Recipient*	Location (City/ Province)	Name of Contact Person	Phone Number	USE:			
				Treatment	Research	Disposal	Unsure
#1				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#2				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#3				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#4				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#5				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

(Attach additional lists if necessary)

* Outside recipients include health care institutions, individual researchers, agencies, disposal companies, funeral homes and/or related organizations, cemeteries, etc.

9.(a) Do you have a written protocol for the handling of ovarian tissue (including its disposal and/or storage)?

Yes ----> Please attach a copy of the written protocol and blank versions of all consent forms used (including blank versions of any consent forms signed by the woman herself)

No ----> Please outline your procedure, including disposal and/or storage of ovarian tissue:

9.(b) How long has this protocol/procedure been in use?

Since: _____ (MONTH/YEAR)

9.(c) Do you anticipate any changes to this protocol/procedure in the near future, say, the next 6 months?

No

Yes ----> Please elaborate:

D. Abortuses/Fetal Tissues

10.(a) What happens to abortuses and/or their tissues in this health care facility? (CHECK AS MANY AS APPLY)

- Not applicable/no abortuses ----> **GO TO QUESTION #12(a), NEXT PAGE**
- Disposed of in-house
- Disposed of through an outside agency, institution or company
- Retained, at least in part, for use in research at this facility
- Provided, at least in part, to other institutions, agencies, companies or individual researchers

10.(b) For each outside recipient, specify:

Name of Outside Recipient* Province)	Location (City/ Province)	Name of Contact Person	Phone Number	USE: (Check all that apply)			
				Treatment	Research	Disposal	Unsure
#1				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#2				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#3				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#4				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#5				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

(Attach additional lists if necessary)

* Outside recipients include health care institutions, individual researchers, agencies, disposal companies, funeral homes and/or related organizations, cemeteries, etc.

11.(a) Do you have a written protocol for the handling of abortuses and/or fetal tissues (including their disposal and/or storage)?

- Yes ----> Please attach a copy of the written protocol and blank versions of all consent forms used (including blank versions of any consent forms signed by the woman herself)
- No ----> Please outline your procedure, including disposal and/or storage of abortuses and/or fetal tissues:

11.(b) How long has this protocol/procedure been in use?

Since: _____ (MONTH/YEAR)

11.(c) Do you anticipate any changes to this protocol/procedure in the near future, say, the next 6 months?

No

Yes ----> Please elaborate:

E. Placentas

12.(a) What happens to placentas in this health care facility? (CHECK AS MANY AS APPLY)

- Not applicable/no placentas ----> **GO TO QUESTION #14, NEXT PAGE**
- Disposed of in-house
- Disposed of through an outside agency, institution or company
- Retained, at least in part, for use in research at this facility
- Provided, at least in part, to other institutions or agencies, companies or individual researchers

12.(b) For each outside recipient, specify:

Name of Outside Recipient*	Location (City/ Province)	Name of Contact Person	Phone Number	USE:			
				(Check all that apply)			
#1				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#2				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#3				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#4				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#5				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

(Attach additional lists if necessary)

* Outside recipients include health care institutions, individual researchers, agencies, disposal companies, funeral homes and/or related organizations, cemeteries, etc.

13.(a) Do you have a written protocol for the handling of placentas (including their disposal and/or storage)?

Yes ----> Please attach a copy of the written protocol and blank versions of all consent forms used (including blank versions of any consent forms signed by the woman herself)

No ----> Please outline your procedure, including disposal and/or storage of placentas:

13.(b) How long has this protocol/procedure been in use?

Since: _____ (MONTH/YEAR)

13.(c) Do you anticipate any changes to this protocol/procedure in the near future, say, the next 6 months?

No

Yes ----> Please elaborate:

14. Do you have any comments on the questionnaire or on new reproductive technologies that you would like to share with the Royal Commission?

THANK YOU FOR YOUR TIME AND PATIENCE

Please be sure to enclose all protocols for handling tissues, related blank consent forms, and all project information, in the return envelope provided.

Finally, please provide us with your name, telephone number and FAX number. Keeping a photocopy of the completed questionnaire for your files would aid in any needed clarification.

Name (PLEASE PRINT): _____

Title: _____

Telephone: (____) _____ - _____

FAX: (____) _____ - _____

Name of Administrator/CEO, etc. if not given above: _____

Title: _____

**RESEARCH PROJECT FORM
(For Health Care Facilities)**

(TO REPORT ON ADDITIONAL PROJECTS,
PLEASE DUPLICATE THIS PAGE AND ATTACH)

R.1 Project #: _____ **R.2 Title:** _____

R.3 Brief Description of Research Project: (attach summary, if available)

R.4 Names and Affiliations of Principal Investigator(s): _____

R.5 Status of Project: (CHECK AND COMPLETE ONLY ONE)

- Completed in the past 12 months
- Now underway (Start Date: _____, Finish Date: _____)
- Planned for the next 12 months
(Start Date: _____, Finish Date: _____)

R.6 What are/were the source(s) and amounts of funding? (Attach additional list if necessary)

(a) Source: _____ Amount: \$ _____

(b) Source: _____ Amount: \$ _____

R.7 **Type of Tissue Used for Research:** (CHECK AS MANY AS APPLY)

Embryos Abortuses and/or fetal tissues Placenta
 Ova Ovarian tissue

R.8 Are/were surplus ova, artificially matured ova, or surplus embryos used, or are embryos created specifically for purposes of research? (CHECK AS MANY AS APPLY)

From Patients in This Health Care Facility	From Outside This Health Care Facility	
<input type="checkbox"/>	<input type="checkbox"/>	Yes, use surplus ova
<input type="checkbox"/>	<input type="checkbox"/>	Yes, use artificially matured ova
<input type="checkbox"/>	<input type="checkbox"/>	Yes, use surplus embryos
<input type="checkbox"/>	<input type="checkbox"/>	Yes, create embryos for research
<input type="checkbox"/>	<input type="checkbox"/>	Yes, other (PLEASE SPECIFY): _____
<input type="checkbox"/>	<input type="checkbox"/>	No, none of the above

R.9 If tissues are/were obtained from outside this facility, for each outside source, specify:

Name of Outside Source*	City/ Province	Name of Contact Person	Area Code and Telephone #
#1			
#2			
#3			
#4			
#5			

(Attach additional lists if necessary)

* Outside sources include health care institutions, individual researchers, agencies, companies, or other organizations.

Survey of Abortion Clinics Regarding Research Uses and Handling of Human Fetal Tissues

INTRODUCTION AND INSTRUCTIONS: The Royal Commission on New Reproductive Technologies needs information regarding a variety of topics including research projects which involve the use of fetal tissues or whole abortuses. We would like to find out about the handling of such tissues or abortuses including their use in research projects now in progress or being actively considered.

In addition, we are interested in information regarding the handling of these tissues other than for research (including sources of and disposal of tissues). This means that many of the questions will apply to your pregnancy termination facility even if there is no research underway. Please be assured that the information you provide will remain confidential. Only aggregated data will be published. Where institutions, agencies, or researchers are identified by you as sources and/or recipients of tissues, and if they have not been contacted through the initial survey, we intend to forward a similar survey to them.

Most of the questions which follow require only a check mark or a brief written answer. Where text answers are requested, please type or complete using ink.

In some cases, we ask you to attach copies of documents (e.g. protocols/consent forms). Please be sure to read each question carefully to determine if it applies to your clinic. *The questionnaire should be completed by the person in your organization most knowledgeable about these procedures.* Please follow the attached "instructions for return", to send the survey to: Survey of Health Care Facilities, Royal Commission on New Reproductive Technologies, 2318 Danforth Avenue, 2nd Floor, Toronto, Ontario, M4C 1K7. If you have any questions, please telephone the survey "hotline" at (416) 467-8430.

Thank you in advance for your time and assistance.

1.(a) What happens to abortuses in this clinic? (CHECK AS MANY AS APPLY)

- Disposed of in-house
- Disposed of through an outside agency, institution or company
- Retained, at least in part, for use in research in this clinic
-----> Please complete attached green Research Project Form
- Provided, at least in part, to other institutions, agencies, companies, or individual researchers

1.(b) For each outside recipient, specify:

Name of Outside Recipient*	Location (City/ Province)	Name of Contact Person	Phone Number	USE:			
				Treatment	Research	Disposal	Unsure
#1				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#2				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#3				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#4				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
#5				<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

(Attach additional lists if necessary)

* Outside recipients include health care institutions, individual researchers, agencies, disposal companies, funeral homes and/or related organizations, cemeteries, etc.

2.(a) Does this clinic have a written protocol for the handling of abortuses (including their disposal and/or storage)?

Yes ----> Please attach a copy of the written protocol and blank versions of all consent forms used (including blank versions of any consent forms signed by the woman herself)

No ----> Please outline your procedure, including disposal and/or storage of abortuses:

2.(b) How long has this protocol/procedure been in use?

Since: _____ (MONTH/YEAR)

2.(c) Do you anticipate any changes to this protocol/procedure in the near future, say the next 6 months?

No

Yes ----> Please elaborate:

3. Do you have any comments on the questionnaire or on new reproductive technologies that you would like to share with the Royal Commission?

THANK YOU FOR YOUR TIME

Please be sure to enclose any protocols for handling abortuses and/or fetal tissues, and related blank consent forms in the return envelope provided.

Finally, please provide us with your name, telephone number and FAX number, in case we need to contact you to clarify any answers. A photocopy of the completed questionnaire for your files would aid in any needed clarifications.

Name (PLEASE PRINT): _____

Title: _____

Telephone: () _____ - _____

FAX: () -

**RESEARCH PROJECT FORM
(For Pregnancy Termination Facilities)**

(TO REPORT ON ADDITIONAL PROJECTS,
PLEASE DUPLICATE THIS PAGE AND ATTACH)

R.1 **Project #:** _____ R.2 **Title:** _____

R.3 **Brief Description of Research Project:** (attach summary, if available)

R.4 **Names and Affiliations of Principal Investigator(s):** _____

R.5 **Status of Project:** (CHECK AND COMPLETE ONLY ONE)

Completed in the past 12 months
 Now underway (Start Date: _____, Finish Date: _____)
 Planned for the next 12 months
(Start Date: _____, Finish Date: _____)

R.6 What are/were the source(s) and amounts of funding? (Attach additional list if necessary)

(a) Source: _____ Amount: \$ _____
(b) Source: _____ Amount: \$ _____

R.7 **Type of Tissue Used for Research:** (CHECK AS MANY AS APPLY)

Abortuses
 Fetal tissues

R.8 If tissues are/were obtained from outside this facility, for each outside source, specify:

Name of Outside Source*	City/Province	Name of Contact Person	Area Code and Telephone #
#1			
#2			
#3			
#4			
#5			

(Attach additional lists if necessary)

* Outside sources include health care institutions, individual researchers, agencies, companies, or other organizations.

Acknowledgments

This study was conducted by SPR Associates, with a team comprised as follows: The study team was led by Dr. Ted Adam Harvey (Project Director), with assistance from Ms Caroline Hunt and Ms Marian Ficycz (Associate Directors), and with assistance from Mr. David Judge, Ms Sylvie Dallari, Ms Mary Smith, and Ms Katarina Pavlovic.

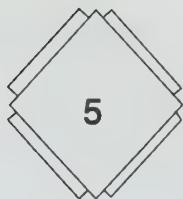
This study benefited greatly from assistance provided by members, staff, and consultants of the Royal Commission on New Reproductive Technologies. In particular, SPR Associates would like to thank Ms Lori Hubbert (for help in the initial stages of the survey), Ms Millie Bilsky (for help in later stages of the study), and many others. SPR would also like to thank Ms Margaret de Groh, who provided assistance in the refinement of the statistical presentations and related text.

The study could not have proceeded at all without the broader cooperation of the many officials of health care facilities and clinics who completed the survey.

Their participation and support in no way indicates agreement with the analysis of conclusions, which is SPR Associates' alone.

Notes

1. SPR conducted no analysis of these materials, but noted for each questionnaire whether such additional materials were attached to the returned questionnaire.
2. A follow-up survey was carried out for the Commission and is also reported in this volume of research.
3. Some caution is warranted here concerning the statistical estimates for the use of ova and embryos, particularly in hospitals offering fertility treatments. This caution is warranted because of missing observations from several hospitals concerning in-house fertility clinics. These hospitals did not report fertility treatments, although in-house clinics are known to exist. This phenomenon was thought by the researchers to be a function of the relatively autonomous nature of some of these clinics within their respective hospitals.



Report on a Follow-Up Survey of Use and Handling of Human Reproductive Tissues (Survey of Medical Laboratories and Medical Waste Disposal Firms)

SPR Associates Inc.



Executive Summary

The objective of this follow-up survey was to provide additional details and to verify and clarify the results of a previous survey on the use and distribution of human reproductive tissues obtained at Canadian health care facilities. That survey was conducted during the period November 1991 to February 1992, with the more than 650 hospitals and other facilities in Canada offering obstetric, gynaecological, and pregnancy-termination services providing data.

This follow-up survey was to clarify the specific use of human reproductive tissues received from these facilities by other institutions, researchers, and agencies. This survey was seen as having particular importance in cases where representatives of health care facilities reported that they were unsure of the use to which tissues were put when distributed outside of the health care facilities.

The survey employed two questionnaires, one for medical laboratories and another for medical waste disposal firms. These questionnaires were generally similar, but the medical laboratories version

contained some additional questions regarding uses for pathology/testing¹ and research.

The survey included a total of 83 organizations drawn from the previous survey of health care facilities. This total consisted of 60 medical laboratories (mostly regional facilities affiliated with larger hospitals and universities) and 23 medical waste firms (including regional offices of larger national firms). As well, an additional 32 organizations not identified as receiving human reproductive tissues by health care facilities (26 laboratories and 6 medical waste firms) were surveyed in order to validate the completeness of reports by health care facilities on distribution of human reproductive tissues.

Survey procedures included an initial mailing of the survey questionnaire by Canada Post Special Letter to all of the organizations surveyed, with subsequent telephone follow-up and faxed reminder letters to non-respondents.

The survey was generally successful for one conducted within a short time frame, with a good response rate overall. Responses were received from 69 of 86 laboratories surveyed (80%), and from 21 of 29 medical waste firms surveyed (72%). Tables within the report show results, however, only for the 48 responding laboratories and 16 responding medical waste firms drawn from the original health care facilities survey, with separate discussion of results for the validation samples.

Medical laboratories identified by the previous health care facilities survey (n = 48) reported uses for a variety of human reproductive tissues: 36 used placentas (75%), 31 used abortuses or fetal tissue (64.6%), 30 used ovarian tissues (62.5%), 19 used embryos (39.6%), and 2 used ova (4.2%). Uses of human reproductive tissues reported were primarily for testing/pathology (34 labs, 70.8%), as was hypothesized in the analysis of the 1991-92 survey of health care facilities. Other uses were research (11 labs, 22.9%, primarily with placentas), disposal (10 labs, 20.8%), and treatment (2 labs, 4.2%). Further distribution of human reproductive tissues was reported in a number of cases, generally for disposal (15 labs), but sometimes for other uses, primarily pathology/testing (6 labs) and research (4 labs). One laboratory reported that it sends placentas to a medical waste firm that collects placentas for commercial use.

As with the previous survey of health care facilities, written protocols for the handling of human reproductive tissues were not found in many cases. Slightly more than half (55%) of laboratories handling human reproductive tissues reported the use of written protocols.

The 48 medical laboratories surveyed reported 19 research projects. The majority of these projects were reported to be funded by the Medical Research Council of Canada, with additional funding from other bodies such as Health and Welfare Canada. None of these projects had been reported in the previous survey of health care facilities that were the sources of the tissues, although often the recipient laboratory in question was affiliated with a major hospital that had responded to the previous survey — the survey source was not aware of the end use in these cases.

Tissues received by medical waste firms identified by the previous health care facilities survey ($n = 16$) were in almost all cases intended for disposal, with medical waste generally being handled on a closed-container basis. The one exception noted is a medical waste firm that makes special provisions for the purchase and collection of placentas for commercial purposes. This firm reported that it receives placentas from over one hundred major Canadian hospitals and redistributes them to Institut Mérieux in Lyon, France, for the production of pharmaceutical products (e.g., vaccines, immunotherapeutic agents). These placentas would otherwise have to be incinerated. They are viewed by the hospitals as waste by-products of childbirth and have been sold to this firm by hospitals for at least the last 20 years.

Uses of human reproductive tissues were generally as expected, with most laboratory-bound human reproductive tissues being used for pathology/testing. Most disposal-bound human reproductive tissues were simply disposed of — with the above-noted exception of placentas.

As with the previous survey of health care facilities, some data quality issues and limitations were noted, including imperfect information on these matters available within health care organizations and the need to examine organizational subdivisions to obtain a complete view of organizational procedures. This need was reflected most clearly in the identification of 19 new research projects from this follow-up survey.

Introduction

Survey Objectives

The objective of the follow-up survey was to clarify results and provide additional details on a previous survey on the use and distribution of human reproductive tissues from Canadian health care facilities. That survey was conducted during the period November 1991 to February 1992 and included over 650 hospitals and other facilities providing obstetric, gynaecological, and pregnancy-termination services in Canada.

A particular purpose of the follow-up survey was to clarify the use of human reproductive tissues in cases where representatives of health care facilities reported that they were unsure of the use to which tissues were put by those institutions, researchers, or agencies receiving them. To provide this clarification, the laboratories and medical waste firms that had been identified by health care facilities as receiving human reproductive tissues were directly approached in a separate follow-up survey. The survey encompassed all laboratories and medical waste firms that could be identified from the data files for the previous survey of health care facilities as recipients of distributed human reproductive tissues.

Purpose of This Report

This report provides additional details that serve to verify and clarify the results obtained in the previous survey of health care facilities, particularly in cases where representatives were unsure of the use to which tissues were put when they were distributed outside their own facilities.

The Survey

The Questionnaires

The survey relied on two questionnaires, one for medical laboratories and another for medical waste disposal firms. These were generally similar, but there were some additional questions in the medical laboratories version regarding uses for pathology/testing and for research. These questionnaires are provided in Appendix 1 of this report.

Survey

The survey examined 83 organizations that had been identified from the 1991-92 survey of health care facilities as recipients of human reproductive tissues. There were 60 medical laboratories (mostly regional facilities affiliated with larger hospitals and universities) and 23 medical waste firms (including regional offices of larger national firms) identified. In addition, another 32 organizations not indicated by health care facilities (26 laboratories and 6 medical waste firms) were surveyed to validate the completeness of reports by health care facilities on the distribution of human reproductive tissues (see Table 1). This validation group was obtained by sampling firms from provincial government lists of medical laboratories in Ontario and Manitoba and by sampling medical waste firms from telephone directories in major urban centres in all provinces.

Table 1. Follow-Up Survey

Source	Organization type	
	Laboratories	Medical waste firms
Health care facilities survey	60	23
Validation sample	26	6
Total	86	29

Survey Procedures

Survey procedures included the following steps:

1. The survey listings were screened to verify addresses, names of contacts, and eligibility for the survey. Some cases were identified where reports from health care facilities appeared to be in error (e.g., an organization was incorrectly identified as receiving human reproductive tissues) and other cases were identified where corporate restructuring — particularly in the medical waste industry — resulted in consolidation of the regional operations of several firms.²
2. An initial mailing of the survey questionnaire by Canada Post Special Letter was made in May 1992 to all of the sampled organizations.
3. Subsequent follow-ups to non-respondents were conducted by fax and telephone throughout June 1992.

Response Rates

The survey was generally very successful for one conducted within a short time frame, with a good response rate. Overall, responses were received from 69 of 86 medical laboratories surveyed (80%), and from 21 of 29 medical waste firms surveyed (72%) (see Table 2). The response rate for laboratories identified through the health care facilities survey was 80% (48 of 60 laboratories surveyed), and the response rate for medical waste firms identified through the health care facilities survey was 70% (16 of 23 firms surveyed). The response rate for the validation group was 81% for medical laboratories (21 of 26 laboratories surveyed) and 83% for medical waste firms (5 of 6 firms surveyed).

Table 2. Response Rates

Source	Organization type			
	Laboratories		Medical waste firms	
	(n)	(%)	(n)	(%)
Health care facilities survey	48	80	16	70
Validation/test sample	21	81	5	83
Total	69	80	21	72

Medical Laboratories: Uses and Distribution of Human Reproductive Tissues

Uses of Tissues

Medical laboratories drawn from the original health care facilities survey reported what was done with a variety of human reproductive tissues. The 48 responding medical laboratories handled the following tissues: placentas (36 labs), abortuses or fetal tissue (31 labs), ovarian tissue (30 labs), embryos (19 labs), and ova (2 labs). The most frequently reported use of human reproductive tissues was testing/pathology, as was hypothesized in the analysis of the previous survey of health care facilities, with 34/48 laboratories doing pathology examination or testing on at least some tissues. Research uses were nine laboratories with placentas, four with ovarian tissue, one with fetal tissue, and one with embryos. The number of laboratories disposing of tissue or using it in treatment is shown in Table 3a. There were no other uses of tissues reported by the responding medical laboratories. Ova were not used for research or treatment by any of these laboratories. See Table 3a for details.

Table 3a. Uses of Human Reproductive Tissues by 48 Medical Laboratories

Tissue type	Use*				
	Pathology/ testing	Disposal	Treatment	Research	All uses
Ova	2	0	0	0	2
Embryos	19	4	1	1	19
Ovarian tissue	28	6	1	4	30
Abortuses or fetal tissue	31	9	2	1	31
Placentas	32	8	1	9	36
At least one tissue type	34	10	2	11	

* Table shows the number of the 48 laboratories reporting use of a given type of tissue. It does not estimate total numbers of ova, embryos, etc. used for each purpose. Multiple uses are possible.

Distribution of Tissues to Other Organizations

The further distribution of human reproductive tissues reported by medical laboratories is summarized in Table 3b. As indicated in this table, 15 medical laboratories passed on various types of human reproductive

tissues for disposal. Four laboratories reported passing on tissues to be used in research, with all four of these laboratories indicating they passed on placentas and embryos for research purposes. Also as indicated in Table 3b, six laboratories reported passing on human reproductive tissues for pathology/testing purposes: five of these six laboratories reported passing on placentas, and two of these six laboratories reported passing on other types of tissues (one passed on embryos and one passed on ovarian tissue). Finally, one laboratory reported sending placentas to the medical waste firm that collects placentas for commercial use (see "Commercial Use of Placentas").

Table 3b. Reasons for Further Distribution of Human Reproductive Tissues

Number of laboratories	Use/reason for distribution
15	disposal
4	research
6	pathology/testing
1	commercial use

Protocols

As with the previous survey of health care facilities, many laboratories did not have written protocols for the handling of human reproductive tissues. Slightly more than half (55%) of the laboratories handling human reproductive tissues reported the use of written protocols.

Research Projects

Laboratories reported 19 research projects. These projects were being conducted at the time of the survey, had recently been completed, or were to begin shortly (see Table 4). More than half of these projects were reported to be funded totally or in part by the Medical Research Council of Canada (MRC), with additional funding of some by Health and Welfare Canada, the National Cancer Institute, the Quebec Ministry of Health, and others. None of these projects had been reported in the previous survey of the health care facilities that were the sources of the reproductive tissues. In many cases the laboratory in question was affiliated with a major hospital that had responded to the previous survey, which again reveals that the person responding on behalf of the parent organization simply did not know about the end use.

The majority of the research projects involve the use of placentas (10 projects). Ovarian tissue is used in three projects (one of them utilizes

tumour cells only); embryos are used in two projects; abortuses/fetal tissue are used in one project; and ova will be used in one upcoming project. The remaining two projects use a combination of tissues: one uses embryos and abortuses/fetal tissue; the other uses embryos, abortuses/fetal tissue, and placentas.

Table 4. Research Projects Reported by Responding Medical Laboratories

Currently being conducted at time of survey:

Plasminogen activators and inhibitors in normal and pre-eclamptic pregnancy
 Role of macrophage cytokines in inflammation
 MRC group in fetal and neonatal health and development
 Embryology of locomotor system
 Studies on human labour
 Biology of the feto-maternal interface
 Characterization of the syncytiotrophoblastic membranes of the human placenta:
 study of the transport of ions
 Immunobiology of ovarian cancer
 Immortalization of human first trimester placental trophoblast cells
 Identification of genes expressed at the early stages of human retinal
 development
 Karyotype-phenotype correlations in human spontaneous abortions
 Human infertility: clinicopathologic study
 Cytokine regulation in the placenta: implications for (congenital) perinatal AIDS

Planned for next 12 months:

Human oocyte freezing

Completed in past 12 months:

Hormonal control of ovarian function
 Localization of 25-hydroxy prostaglandin dehydrogenase in human fetal
 membranes, decidua, and placenta during pregnancy
 Regulation of prostaglandin synthesis in the human amnion
 Localization and distribution of corticotropin-releasing hormone in the human
 placenta and fetal membranes throughout gestation
 Evidence that fibronectin in human placentas is derived from intermediate
 trophoblasts

Validation Sample

The 26 laboratories sampled to test the accuracy of health care facilities reports in the previous survey generally supported the validity of the previous survey, with only one of these laboratories reporting any handling of human reproductive tissues.

Medical Waste Firms and Human Reproductive Tissues

Provincial regulations require all potentially pathological human waste to be sealed in special containers. As a result, most biomedical waste firms indicated that they were unable to confirm exactly what type of waste was sent to them. In almost all cases, the tissues received by medical waste firms were simply disposed of, generally being handled on a closed-container basis.

Commercial Use of Placentas

One particular medical waste firm was identified as illustrating a major exception to closed-container disposal. In addition to its other activities, this firm purchases and collects placentas from hospitals for commercial uses. It sends all of these placentas to the Institut Mérieux in Lyon, France.

This Canadian firm purchases placentas from over one hundred major Canadian hospitals. The placentas are then passed on to the Institut Mérieux, through the Institut Mérieux agent in the United States. The placentas are used in the production of a variety of pharmaceutical products, including human albumin, polyvalent immunoglobulins for intramuscular use (gamma 16); polyvalent immunoglobulins for intravenous use (veinoglobulin); histamine-protective immunoglobulin (allerglobulin); and glucocerebrosidase (G.C.R.).

Worldwide, Institut Mérieux deals with more than 8 000 maternity facilities, processing 3 000 to 4 000 tons of placentas per year. Canada's portion is estimated at 40 to 60 tons. At present, all of the human placentas exported from Canada by the identified medical waste firm are reported to go to the Institut Mérieux. The amount hospitals receive per placenta is reported to be approximately \$0.35.

Some health care facilities dealing with this Canadian medical waste firm were not sure about the precise end use of the tissues in their responses to the original survey. When the data from the original health care facilities providing placentas to this firm were re-examined, it was found that the respondents had variously indicated that the end use was treatment, research, production of pharmaceuticals, production of cosmetics, disposal, or other specific uses (see Table 5). The largest group (31%) had reported that they were unsure of the end use.

As far as the survey determined, Institut Mérieux uses human placentas for pharmaceutical purposes only. Although there are cosmetics in Canada labelled as containing "human placental extract," there is no evidence at this time that human placentas from Canada are being used for cosmetic purposes.

Some controversy surrounds the selling of placentas, and the lack of patient consent for this use of placentas ("Province to Ban Sale of Placentas by Hospitals," *Montreal Gazette*, December 30, 1991; and "Woman Upset

Hospitals Selling Placentas," *Saskatoon Star Phoenix*, June 25, 1992). Hospitals view these as waste by-products of childbirth, which they would otherwise have to pay to have incinerated.

Table 5. Reported Uses of Human Reproductive Tissues by Health Care Facilities Selling Placentas to One Medical Waste Firm*

Reported use	%	n**
Unsure	31	22
Treatment	24	17
Research	18	13
Disposal	16	11
Other (e.g., production of gamma globulin and other pharmaceuticals)	10	7

* Data are taken from health care facilities survey.

** Based on reports from 70 hospitals. While only 70 hospitals reported the sale of placentas to this medical waste firm, the firm purchasing the placentas reported receiving this material from over one hundred major Canadian hospitals.

Protocols

A variety of protocols were identified by medical waste companies, mostly in relation to provincial regulations for handling waste. These generally described systems for clearly packaging and labelling "hazardous" medical waste. The medical waste firm that purchases placentas provides hospitals with a protocol for collecting placentas and placental blood.

Validation Sample

None of the additional five medical waste firms surveyed for validation purposes reported that they handled human reproductive tissues, supporting the accuracy of previous health care facilities reports.

Discussion: Medical Waste Component

The follow-up survey of medical waste firms identified the large number of Canadian hospitals supplying placentas to the one commercial firm and indicated the end use of these materials as being for pharmaceutical purposes. It also made evident the fact that many health care facility administrators are not fully informed about the specific end use of placentas from their hospitals.

Conclusions

Uses of human reproductive tissues were generally as expected. Most laboratory-bound human reproductive tissues undergo pathology/testing. Those tissues going to medical waste firms were simply being disposed of, with the one exception of placentas.

Some data quality issues and limitations were revealed, including imperfect information within health care facilities and the need to examine organizational subdivisions to obtain complete information.

The placentas sold by health care facilities are forwarded by the medical waste firm receiving them to the Institut Mérieux in France for production of pharmaceutical products such as vaccines, gamma globulin, and other therapeutic agents. The Institut Mérieux recently acquired Connaught Laboratories in Toronto as one of its initiatives in developing new vaccines.

Provincial human tissue gift acts in Canada forbid the selling of human tissues for medical or therapeutic research. It is evident that placentas are not viewed by these hospitals as coming within the acts but are seen as a waste by-product of childbirth and are classified as "discarded human body material" that would otherwise have to be incinerated. As discussed, cases were noted where health care facilities indicated incorrectly that the use of placentas was disposal (see Table 5).

Appendix 1. Survey Questionnaires

Survey of Medical Laboratories Regarding Use/Distribution of Human Reproductive Tissues

INTRODUCTION AND INSTRUCTIONS: In accordance with its mandate, The Royal Commission on New Reproductive Technologies requires information regarding a variety of topics dealing with the use/distribution of human reproductive tissues.

This survey of medical laboratories is an important part of this work, complementing a previous survey of health care facilities offering obstetric and gynecological services. Your response to this survey is needed regardless of whether or not your organization deals with human reproductive tissues in any way. Please review the questions carefully in order to respond to the specific questions that apply to your organization.

Please be assured that the information you provide will remain *strictly confidential*. Only aggregated data will be published. Institutions, agencies, or researchers identified by you as sources and/or recipients of tissues will not be revealed in any way. Most of the following questions require only a check mark or a brief written answer. In some places, when you choose an answer, you are asked to skip to another question further on. Please follow these instructions. Where text answers are requested, please **type**, or **print** using ink. In some cases, we ask you to attach copies of documents (e.g., protocols/consent forms). Please be sure to attach these documents even if you have provided them already to the Commission in connection with other projects/surveys. Be sure to read each question carefully to determine how the question applies to your organization.

The questionnaire should be completed by the person in your organization who is most knowledgeable about these matters. Please return the survey within the next week, preferably by FAX, to: (416) 467-0517, or by mail to: Survey of Medical Laboratories, Survey Office, Royal Commission on New Reproductive Technologies, 2318 Danforth Avenue, 2nd Floor, Toronto, Ontario, M4C 1K7. If you have any questions, please telephone the survey "hotline" at (416) 467-8430.

Thank you in advance for your time and assistance.

A. Receipt and Use of Human Reproductive Tissues

1. In the past 12 months, has your organization ever received, handled, or distributed any of the following human reproductive tissues from hospitals, clinics or other organizations? (CHECK ALL THAT APPLY)

- Ova
- Embryos
- Ovarian tissues
- Abortuses/Fetal tissues
- Placentas
- None of the above is ever received or used by this laboratory, or distributed from this laboratory ---> (GO TO SECTION D, LAST PAGE)

2. For what purposes does your organization use human reproductive tissues? (INDICATE EACH USE THAT APPLIES TO EACH TYPE OF TISSUE USED OR HANDLED IN YOUR ORGANIZATION)

USES OF TISSUES (CHECK ALL THAT APPLY, FOR EACH TYPE OF TISSUE)

TYPES OF TISSUES	In-house Disposal Only	Testing/ Pathology	Medical Treatment	Research	Other Uses	If you have indicated "other uses", please provide details below:
						□
(a) Ova	<input type="checkbox"/>	—				
(b) Embryos	<input type="checkbox"/>	—				
(c) Ovarian tissues	<input type="checkbox"/>	—				
(d) Abortuses/Fetal tissues	<input type="checkbox"/>	—				
(e) Placentas	<input type="checkbox"/>	—				

→ If you have indicated a research project use, please complete one copy of the attached blue research project form for each project.

3. Are there written procedures or protocols/guidelines for handling human reproductive tissues within your organization?

□ No

Please attach (including blank copies of any standard consent forms/agreements, if you deal directly with individuals)

B. Sources of Human Reproductive Tissues

4. If you have received any human reproductive tissues, please indicate below, the source from which they were received (including other departments or branches of your own organization), and the types of tissues received. (COMPLETE ONE LINE FOR EACH SPECIFIC SOURCE FROM WHICH HUMAN REPRODUCTIVE TISSUES WERE RECEIVED)

Please photocopy this page if you need additional space to report on sources of human reproductive tissues, and attach.

C. Distribution of Human Reproductive Tissues

5. Have you distributed any human reproductive tissues to other organizations for any purpose in the past year, including disposal?

Yes No ---> (GO TO SECTION D, NEXT PAGE)

6. If you have distributed any human reproductive tissues, please indicate below, the recipients of these tissues and the types of tissues distributed. (COMPLETE ONE LINE FOR EACH SPECIFIC RECIPIENT ORGANIZATION TO WHICH HUMAN REPRODUCTIVE TISSUES WERE DISTRIBUTED).

* To specify "use" made by recipient, please code: "D" for disposal; "R" for research; "C" for commercial use; "O" for other; and "U" for unknown.

If “other”, please explain:

Please photocopy this page if you need additional space to report on organizations to which you have distributed human reproductive tissues, and attach.

D. Survey Completion

7. If you have any comments on the questionnaire or on new reproductive technologies that you would like to share with the Royal Commission, please provide them below.

8. Please attach any protocols you have for handling human reproductive tissues, any related blank consent forms, and all project information, in your FAX or in the return envelope provided. Finally, please provide us with your name, telephone number and FAX number so that we may contact you, if necessary, for clarification. Keeping a photocopy of the completed questionnaire for your files would aid in any needed clarification.

9. Name (PLEASE PRINT): _____ Title: _____
Telephone: (_____) _____ - _____ FAX: (_____) _____ - _____
Name of manager/CEO, etc. if not given above: _____ Title: _____

THANK YOU FOR YOUR TIME AND ASSISTANCE

RESEARCH PROJECT FORM

1. Project Title: _____
2. Brief Description of Research Project: (ATTACH SUMMARY, IF AVAILABLE)

3. Names and Affiliations of Principal Investigator(s):

4. Status of Project: (CHECK AND COMPLETE ONLY ONE)
 Completed in the past 12 months
 Now underway (Start Date: _____, Finish Date: _____)
 Planned for the next 12 months (Start Date: _____, Finish Date: _____)
5. What are/were the source(s) and amounts of funding? (ATTACH ADDITIONAL LIST IF NECESSARY)
(a) Source: _____ Amount: \$ _____
(b) Source: _____ Amount: \$ _____
6. Types of Tissues Used for Research: (CHECK AS MANY AS APPLY)
 Ova
 Embryos
 Ovarian tissues
 Abortuses and/or Fetal tissues
 Placentas

(TO REPORT ON ADDITIONAL PROJECTS, PLEASE DUPLICATE THIS PAGE AND ATTACH)

Survey of Medical Waste Companies Regarding Use/ Distribution of Human Reproductive Tissues

INTRODUCTION AND INSTRUCTIONS: In accordance with its mandate, The Royal Commission on New Reproductive Technologies requires information regarding a variety of topics dealing with the use/distribution of human reproductive tissues.

This survey of medical waste companies is an important part of this work, complementing a previous survey of health care facilities offering obstetric and gynecological services. Your response to this survey is needed regardless of whether or not your company deals with human reproductive tissues in any way. Please review the questions carefully in order to respond to the specific questions that apply to your organization.

Please be assured that the information you provide will remain *strictly confidential*. Only aggregated data will be published. Institutions, agencies, or researchers identified by you as sources and/or recipients of tissues will not be revealed in any way. Most of the following questions require only a check mark or a brief written answer. In some places, when you choose an answer, you are asked to skip to another question further on. Please follow these instructions. Where text answers are requested, please **type, or print** using ink. In some cases, we ask you to attach copies of documents (e.g., protocols/consent forms). Please be sure to attach these documents even if you have provided them already to the Commission in connection with other projects/surveys. Be sure to read each question carefully to determine how the question applies to your organization.

The questionnaire should be completed by the person in your organization who is most knowledgeable about these matters. Please return the survey within the next week, preferably by FAX, to: (416) 467-0517, or by mail to: Survey of Medical Waste Companies, Survey Office, Royal Commission on New Reproductive Technologies, 2318 Danforth Avenue, 2nd Floor, Toronto, Ontario, M4C 1K7. If you have any questions, please telephone the survey "hotline" at (416) 467-8430.

Thank you in advance for your time and assistance.

A. Receipt and Use of Human Reproductive Tissues

1. In the past 12 months, has your organization ever received, handled, or distributed any of the following human reproductive tissues from hospitals, clinics or other organizations? (CHECK ALL THAT APPLY)

- Ova
- Embryos
- Ovarian tissues
- Abortuses/Fetal tissues
- Placentas
- None of the above is ever received or used by this company or distributed from this company --> (GO TO SECTION D, LAST PAGE)

2. For what purposes does your organization use human reproductive tissues? (INDICATE EACH USE THAT APPLIES TO EACH TYPE OF TISSUE USED OR HANDLED IN YOUR COMPANY)

USES OF TISSUES (CHECK ALL THAT APPLY, FOR EACH TYPE OF TISSUE)

TYPES OF TISSUES	In-house Disposal Only	Other Uses	If you have indicated "other uses", please provide details below.
(a) Ova	<input type="checkbox"/>	<input type="checkbox"/>	
(b) Embryos	<input type="checkbox"/>	<input type="checkbox"/>	
(c) Ovarian tissues	<input type="checkbox"/>	<input type="checkbox"/>	
(d) Abortuses/Fetal tissues	<input type="checkbox"/>	<input type="checkbox"/>	
(e) Placentas	<input type="checkbox"/>	<input type="checkbox"/>	

3. Are there written procedures or protocols/guidelines for handling human reproductive tissues within your organization?

No Yes --> Please attach (including blank copies of any standard consent forms/agreements, if you deal directly with individuals)

B. Sources of Human Reproductive Tissues

4. If you have received any human reproductive tissues, please indicate below, the source from which they were received (including other divisions or branches of your own organization), and the types of tissues received. (COMPLETE ONE LINE FOR EACH SPECIFIC SOURCE FROM WHICH HUMAN REPRODUCTIVE TISSUES WERE RECEIVED)

Please photocopy this page if you need additional space to report on sources of human reproductive tissues, and attach.

C. Distribution of Human Reproductive Tissues

5. Have you distributed any human reproductive tissues to other organizations for any purpose in the past year, including disposal?

Yes No ---> (GO TO SECTION D, NEXT PAGE)

6. If you have distributed any human reproductive tissues, please indicate below, the recipients of these tissues and the types of tissues distributed. (COMPLETE ONE LINE FOR EACH SPECIFIC RECIPIENT ORGANIZATION TO WHICH HUMAN REPRODUCTIVE TISSUES WERE DISTRIBUTED)

* To specify use made by recipient, please code: "D" for disposal; "R" for research; "C" for commercial use; "O" for other; and "?" for unknown.

If “other”, please explain:

Please photocopy this page if you need additional space to report on organizations to which you have distributed human reproductive tissues, and attach.

D. Survey Completion

7. If you have any comments on the questionnaire or on new reproductive technologies that you would like to share with the Royal Commission, please provide them below.

THE JOURNAL OF CLIMATE

8. Please attach any protocols you have for handling human reproductive tissues, any related blank consent forms, and all other related information, in your FAX or in the return envelope provided. Finally, please provide us with your name, telephone number and FAX number so that we may contact you, if necessary, for clarification. Keeping a photocopy of the completed questionnaire for your files would aid in any needed clarification.

9. Name (PLEASE PRINT): _____

Telephone: () FAX: ()

Name of manager/CEO, etc. if not given above: _____ Title: _____

THANK YOU FOR YOUR TIME AND ASSISTANCE

Acknowledgments

This study was conducted by SPR Associates, with a team comprised as follows: The study team was led by Dr. Ted Adam Harvey (Project Director), with assistance from Ms Caroline Hunt and Ms Marian Ficycz (Associate Directors), and with assistance from Ms Sue Langton (Senior Consultant), Mr. David Judge, Ms Vera Nunes, Ms Mary Smith, and Ms Katarina Pavlovic.

This study benefited greatly from assistance provided by members, staff, and consultants of the Royal Commission on New Reproductive Technologies. SPR Associates would like to thank Ms Millie Bilsky and many others for their help. SPR would also like to thank Ms Margaret de Groh, who provided assistance in the refinement of the statistical presentations and related text.

The study could not have proceeded at all without the broader cooperation of the many officials of the various laboratories and waste management firms who completed the survey.

Their participation and support in no way indicates agreement with the analysis of conclusions, which is SPR Associates' alone.

Notes

1. Note that while these uses were treated together in the survey, the Royal Commission on New Reproductive Technologies has made a distinction between "pathology" and "testing." "Pathology" refers to the evaluation of tissues for disease, in particular the structural and functional changes that result from disease. "Testing" is a broader term; tissues can be tested for both pathological and non-pathological conditions.
2. In particular, two major national medical waste firms merged during the period between the original health care facilities survey and the follow-up survey. This needs to be kept in mind in any comparison of the previous health care facilities reports with the current reports by medical waste firms.

Embryo Transfer and Related Technologies in Domestic Animals: Their History, Current Status, and Future Direction, with Special Reference to Implications for Human Medicine

K.J. Betteridge and D. Rieger



Executive Summary

This study reviews embryo research in the fields of animal husbandry and veterinary medicine and draws parallels to human embryo research.

Technical aspects of reproductive manipulation may be similar for humans and domestic animals, but the objectives are quite different. With humans the purpose of reproductive manipulation is to benefit the individual, whereas artificial insemination (AI) and embryo transfer (ET) in domestic animals are done to improve production, which benefits farmers and, ultimately, consumers. Reproductive manipulations are practised in all domestic animal species; however, work with dairy cattle has been the most extensive.

The rate of genetic gain in domestic animals will increase even more rapidly with the implementation of sophisticated breeding strategies, such as the development of nucleus herds of elite animals through multi-ovulation ET programs. Use of gene transfer to produce animals with new characteristics (e.g., lactose-free milk) is under

investigation. In addition, ET and other techniques may be the only means to preserve certain endangered animal species.

The study lists common areas of interest between medical and veterinary aspects of embryo technology where exchange of information is beneficial. For example, many of the pioneers of *in vitro* fertilization (IVF) developed their expertise with animals. However, the extent to which any technique is transferred from animal to human application, especially those involving genetic manipulation, is governed by factors other than technical feasibility.

The study concludes that advances in our knowledge of comparative reproductive biology resulting from the study of new reproductive technology will be beneficial to society in the long run. Investigation of the mechanisms controlling differentiation in cells during embryonic development will provide fundamental knowledge that will be invaluable for the fields of biology and medicine.

Introduction

The primary purpose of this study is to obtain an understanding of embryo research being pursued in the fields of animal husbandry and veterinary medicine as it relates, or may relate, to human embryo research. Much of the discussion focusses on *in vitro* techniques of embryo manipulation such as *in vitro* fertilization (IVF) and embryo manipulation, which are generally equated with the "new reproductive technologies." We have, however, also included discussion of related techniques such as stimulation of ovarian function, cryopreservation of gametes and embryos, and management of early pregnancy, since as well as being of significant interest in animal work, these subjects are closely related to analogous interests in human medicine.

The information presented in this report is drawn mostly from the published scientific literature. However, it is important to state that the references cited have been selected to provide the reader with a lead into the wider literature concerned with a given topic; they do not necessarily reflect the scientific "priority" that should be credited for the development of particular procedures. In addition to subscribing to several of the major journals in reproductive biology, we maintain up-to-date personal bibliographic data bases, principally by using the computer disk form of *Current Contents*. To ensure that our information was as comprehensive as possible, we also searched the literature using MEDLINE, a public data base of the medical science literature. Both before and since the contract for this report was made, we have attended and participated in specialized research symposia on various aspects of embryo biology. Notable examples are the Serono Symposium on Preimplantation Embryo Development (Boston, Massachusetts, August 1991), an International Symposium on Animal Biotechnology (Kyoto, Japan, October 1991), the annual meetings of the Canadian Association of Animal Breeders (Niagara-on-the-Lake,

Ontario, September 1991) and the International Embryo Transfer Society (Denver, Colorado, January 1992), and the Symposium on Cloning Mammals by Nuclear Transplantation (Fort Collins, Colorado, January 1992). We have also consulted other scientists on specific aspects of their work.

Although the technical aspects of reproductive manipulation of humans and domestic animals are similar, the reasons for using them are obviously different. In humans, the benefit to the individual is the major aim of any manipulation of reproduction. In domestic species, reproduction is almost always manipulated in groups of animals, and done to benefit humans. For example, artificial insemination (AI) and embryo transfer (ET) are widely used in cattle to extend the genetic contribution of superior individuals to the general population; the key concern is not for the individual animal, but to improve the production of the population to benefit the producer and the consumer. An important corollary of this is that most new reproductive technologies in domestic animals aim to produce as many offspring as possible from each manipulation of a selected animal or group of animals; the aim in humans is to maximize the probability of obtaining a single pregnancy from a given manipulation. Keeping in mind these basic differences in the aims of reproductive manipulation of humans and domestic animals should be useful in avoiding any risk of anthropomorphism. This is not always easy; even to describe an animal as a "donor" of eggs or embryos is anthropomorphic, an inappropriate transfer of approach.

Reproductive manipulations are practised to some extent in all domestic species, but most extensively and successfully in dairy cattle. In particular, the widespread use of AI and, more recently, ET, in conjunction with sophisticated methods of milk recording and genetic analysis, has markedly improved milk production in the Canadian Holstein, such that this breed now ranks among the best dairy animals in the world. The relevance of this fact to the Canadian economy is reflected in a recent publication of the Science Council of Canada (1991), which describes the agriculture and food industry as a cornerstone of the Canadian economy. Annual sales exceed \$50 billion and the sector provides direct and indirect employment for 14 percent of the country's labour force. In all, agriculture and food production account for as much as one-third of Canada's trade surplus. Furthermore, within the industry, cattle and dairy products are the leading sources of farm cash receipts, ahead of wheat, which is popularly imagined to be Canada's "cash cow" (*ibid.*). Export of Canadian bovine semen in 1989 was valued at \$30 million. Bovine embryo export is still a relatively minor source of revenue (12 000 embryos valued at \$6 million in 1989), but the potential of this trade is underlined by the willingness of the industry to be very supportive of research in the area and to sponsor, for example, four industrial research chairs at Guelph and

Laval universities in partnership with the Natural Sciences and Engineering Research Council.

Although modern breeding techniques have similar potential to improve the management and economic returns in other domestic animals, applications in them are, as yet, much more limited. Consequently, much of the following discussion will focus on the application of techniques of reproductive management and manipulation to cattle.

As in human medicine, the application of techniques of reproductive manipulation of domestic animals must meet the most basic principle of good medical practice — that the treatments be efficacious and not debilitating. Consequently, veterinary drugs are regulated by the Food and Drug and Narcotic Control Acts and Regulations, and are subjected to rigorous testing before being approved for use. Similarly, standards of practice and professional accreditation for procedures such as ET are being established by the Canadian Embryo Transfer Association, the International Embryo Transfer Society, and other organizations. International movement of gametes and embryos is strictly regulated by national governments (e.g., Agriculture Canada and the U.S. Department of Agriculture) for reasons of disease control. In combination, these factors make the reproductive manipulation of animals subject to considerable constraint, perhaps even more than in human medicine. For example, with many veterinary pharmaceuticals, regulations forbid the sale of meat or milk for consumption before a set minimum withdrawal period to avoid passing the drugs to the human consumer.

Reproductive manipulation is also limited by breed organizations to extents that differ between species, and between breeds within a species. For example, in cattle and quarter horses, purebred offspring arising from either AI or ET can be registered. On the other hand, only limited AI is acceptable in standardbred horses, and no reproductive intervention of this kind is permitted in thoroughbreds. Finally, concerns for animal welfare must increasingly be considered in reproductive manipulation. As a case in point, repeated oocyte recovery by transvaginal follicular puncture may be unlikely to be approved in domestic animals at any Canadian research institution.

Evidently, then, there are important differences to be considered in comparing uses of new reproductive technologies in humans and animals. There are also inescapable similarities, two of which depend on the gametes and embryos themselves, that have important implications for informed discussion of issues being addressed by this Commission. The first, of course, is the fundamental similarity of gametes and early embryos among mammals. One effect of this is to inspire awe and respect in those who work with them. For embryos, this is illustrated by the quotation from Warwick and Berry (1949) cited in Figure 1 (Betteridge 1977). For gametes, a similar respect for even the spermatozoon itself is very evident in a much more recent publication (Hunter et al. 1991). The second similarity, also fundamental, is that the study of fertilization and early embryonic

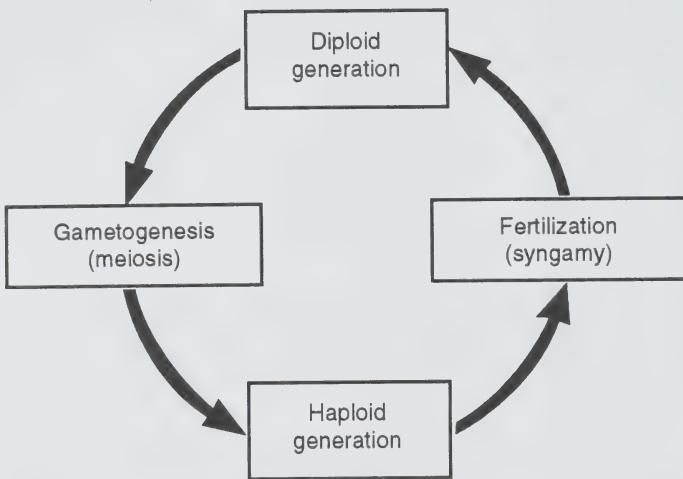
development is probably the most forceful way to be reminded that life has no beginning and no end but is a continuous alternation of haploid and diploid generations (Figure 2). This has been discussed in the context of reproductive manipulation by Biggers (1983, 1990), but popular writers have put it cogently, too. "A hen is an egg's way of making another egg" was Samuel Butler's line in 1877 (from Betteridge et al. 1989b); more recently, Laurens van der Post explained (during a 1986 interview with Vicki Gabereau on CBC radio) that, when he looked at a baby, he thought not of how young it was, but how old. This brings us to the subject of how young, or how old, are the so-called "new" reproductive technologies.

Figure 1. Attitudes Evoked by Mammalian Embryos

There can be few who have examined embryos through the microscope without being excited. Today's ascetic editors leave little room for the expression of feelings, but in any case it is doubtful whether a day of excitement in the laboratory could be any better described than was 8 December 1932:

Five days after we had seen our first living ovine egg, we operated on a goat which had been bred two days previously. We examined both ovaries for corpora hemorrhagica and found one typical corpus. We then removed the corresponding oviduct and proceeded to attempt to find the egg. We were successful; and soon were gazing in awe at a beautiful four-celled goat in saline under the binocular. We wondered why we had been so skeptical of our abilities, and regretted that we did not have a recipient animal of either species ready to become the uterine foster parent of this handsome animal. This animal seemed so beautiful to us that we could not bear to let it die of neglect, so we hastily decided to put it back in its original home for five months. It was picked up in a 20-gauge needle and injected into the uterus of its mother. When, 147 days later, we again looked at this same animal after normal parturition, a good many changes had taken place. (Warwick and Berry 1949, 300-301, reprinted with permission of Oxford University Press)

In the broadest sense, humans have manipulated the reproduction of their domestic animals since prehistoric times. Then, as now, the major approach was to selectively breed from individuals with desirable characteristics and to cull the undesirable. However, the origins of modern reproductive manipulation in animals can, in several ways, be said to

Figure 2. The Alternation of Generations

Source: Betteridge et al. 1989b.

originate with the experiments of Walter Heape, who performed the first successful transfer of mammalian embryos in 1891, rekindled interest in AI, and coined the terminology still used in describing female reproductive cycles. These and other contributions of this remarkable man to reproductive biology were recently summarized at a symposium commemorating the centennial of that first embryo transfer (Biggers 1991). However, many years elapsed between the initial rabbit work and the birth of the first calf from embryo transfer (Willett et al. 1951), and the publication that led to the commercial use of ET in North America (Rowson et al. 1969).

The influence of L.E.A. Rowson and his colleagues in their Cambridge laboratories in the 1960s and 1970s was enormous; scientists who trained there have become leaders in both the animal and human embryo fields throughout the world, and their short courses were instrumental in founding the first commercial ET companies (see Rowson 1976; Carmichael 1980). The 22-year history of commercial ET is of special interest and

relevance in a Canadian context. This is because the incentive to transform a laboratory procedure into a commercially feasible breeding technique was provided by a high demand for European beef cattle breeds in North America beginning in the latter half of the 1960s. The demand, which was based on the apparently superior performances of these breeds, could only be met through Canada because the United States would not permit direct importation of European cattle for fear of introducing foot and mouth disease with them. Agriculture Canada, however, made it possible to import animals by setting up the necessary elaborate screening procedures and quarantine stations, the cost of which added to the prices paid for the relatively low numbers of animals that could pass through them. The possibility of using ET to produce larger numbers of progeny from these beef cattle than would be possible by natural breeding became potentially lucrative and led to the founding of three commercial ET companies in Canada in 1971 (Church 1987; Church and Shea 1976; Shea et al. 1976; Mapletoft 1984). At the same time, Agriculture Canada began its own research into the subject (Betteridge 1977). Apart from a short-lived unsuccessful venture in Texas in 1949 (Betteridge 1981), these ET companies were the first in the world. The only survivor of the three, Alberta Livestock Transplants (recently amalgamated with Western Breeders Services to form Alta Genetics), is the world's oldest and largest ET and manipulation facility, and is still at the centre of trade in embryos of beef breeds, which is about equal in volume to that of dairy breed embryos in Canada. In relation to the parallels and interfaces between animal and human embryo work, it is also of interest that the founders of Alberta Livestock Transplants included a professor of medical biochemistry, Dr. R.B. Church.

More general historical aspects of ET have been summarized by Betteridge (1981), Mapletoft (1984), and Church (1987), while details of pioneering efforts in specific subjects are to be found in the citations that have accompanied the presentation of the International Embryo Transfer Society's "Pioneer Awards." Since 1984, these have been published in the January issues of *Theriogenology* for L.E. Casida, 1984; L.E.A. Rowson, 1985; T. Sugie, 1986; E.J.C. Pole, 1987; A. McLaren and D. Michie, 1988; C. Thibault, 1989; J.D. Biggers, 1990; A.K. Tarkowski, 1991; and R.L. Brinster, 1992.

M.C. Chang's entertaining account of his own work with eggs (1983) illustrates the important overlaps that exist between work with animals and humans and between the fundamental and applied aspects of embryology; while Chang is best known in the present context as the first person to produce live young after IVF, the wider world remembers him (with Pincus) as the inventor of the contraceptive pill. Similarly, while the world knows R.G. Edwards (with P.C. Steptoe) in connection with his role in bringing about the birth of Louise Brown, his contributions to fundamental aspects

of mammalian embryology are equally important (see *Human Reproduction* 1991).

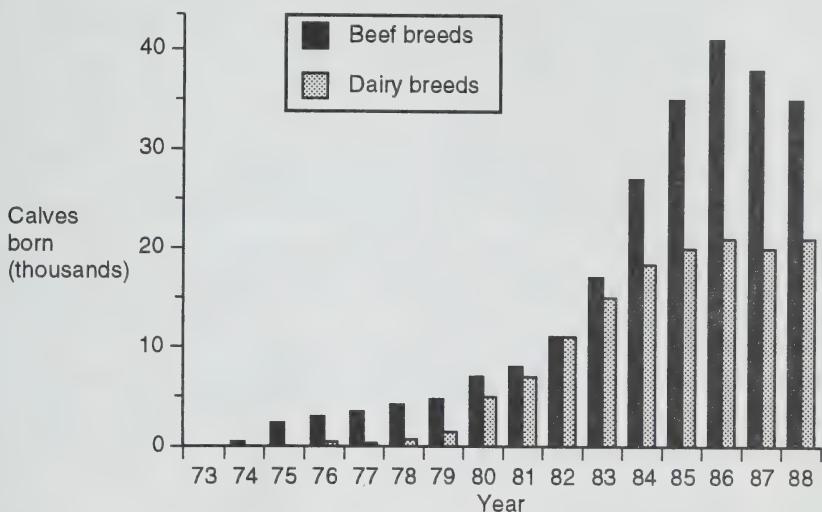
It might seem more appropriate to discuss the use of ET and related procedures as tools in animal production than as novel techniques with their own intrinsic interests. Yet it is the potential power of the related procedures, which depend on ET but are growing so rapidly as a result of parallel advances in molecular biology and developmental embryology, that makes it imperative to describe the techniques that make the tool. Some of the techniques (e.g., cryopreservation) are, by now, well known and are used every day in what can be called the "routine" practice of animal ET, especially in cattle. Others, such as sexing, "cloning," and IVF, have yet to make the transition from the laboratory to the field except in a limited number of commercial companies with their own highly specialized technical staff. Still other techniques, including the making of transgenic animals, need much more laboratory study before their applicability in animal production can be properly assessed. The breadth of this spectrum of technical development strongly suggests that the practice of ET as it exists today may be expected to change as the ET industry comes of age.

Today, the successful practitioners of ET are divided between small and large organizations. Small organizations, most often veterinarians in private practice, depend for their success on meticulous, personal attention to all phases of the chain of events involved in ET, and on a market for their services. Large organizations are far less numerous and include private livestock companies and those linked to AI enterprises, which may be privately or cooperatively owned.

As shown in Figure 3, ET in cattle in North America has increased exponentially since its inception in the early 1970s, reaching more than 60 000 calves registered in the United States in 1988, and 7 000 Holstein calves in Canada alone in 1989. The extent of ET in Europe is indicated by the results of the 1989 survey summarized in Table 1. In the 15 countries surveyed, 25 858 cattle were induced to superovulate, yielding a total of 132 222 good quality embryos, an average of 5.1 per donor cow. In the same year, over 100 000 cattle embryos were transferred to recipients, and 25 277 were held frozen. The contribution of ET to genetic improvement is greater than is suggested by the absolute scale of its use in cattle. Although the calves produced by ET represented only 4 percent of all Holsteins registered in the United States in 1987, they represented the top 27.5 percent of cows and top 44 percent of bulls tested for productivity in 1990 (*Holstein World* 1991, 108). In our own country, of the 465 Canadian Holstein sires with semen available in January 1992, 260 (55.9 percent) were themselves produced by ET from a selected superovulated donor cow inseminated with frozen-thawed semen from a selected sire as a "contract mating." The proportion of the next generation of sires (1987 AI sire entries) that were produced in this way is 175 out of 279 (62.7 percent). The reason for this is that producing several calves from a single such "contract mating" increases the chances of obtaining the required bull-calves.

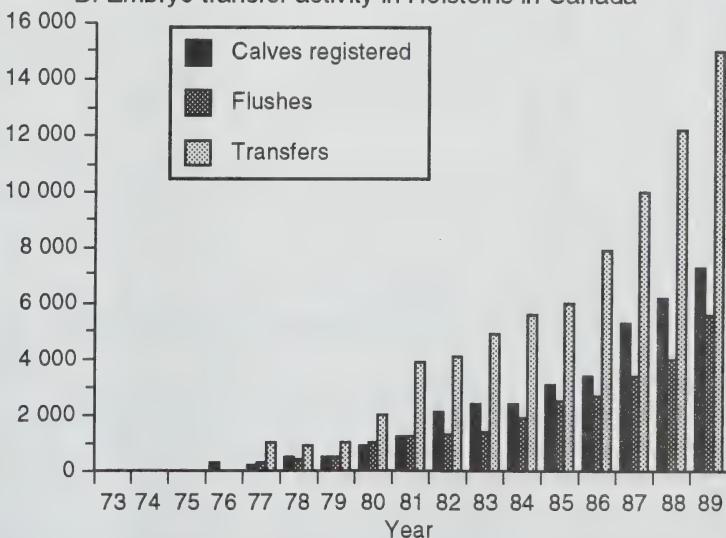
Figure 3. Patterns of Growth in Embryo Transfer in Cattle in North America Since Commercial Activity Began in the Early 1970s

A. Embryo transfer activity in cattle in the United States



Source: Redrawn from the data of Baker 1989.

B. Embryo transfer activity in Holsteins in Canada



Source: From the data obtained through the courtesy of the Holstein Association of Canada.

Table 1. Embryo Transfer Activity Reported in Cattle in Europe for the Year 1989

Country	Donors flushed (% treated donors)	Transferable embryos collected		Embryos transferred		Embryos in frozen storage	
		Total (% total collected)	Per flushed donor	Domestic			
				Fresh (%)	Frozen (%)		
Germany (DDR)	1 496	(83.9)	9 107 (70.9)	6.1	7 547 (100)	—	886
Germany (FRG)	3 570	(94.4)	20 194 (59.5)	5.7	11 713 (66.7)	5 851 (33.3)	7 072
Belgium	582	(94.6)	2 371 (55.2)	4.1	1 229 (56.0)	967 (44.0)	674
Denmark	1 164	(91.2)	7 630 (65.0)	6.6	2 765 (74.4)	953 (25.6)	41
Spain	261	(90.0)	1 090 (62.6)	4.2	453 (68.3)	210 (31.7)	13
Finland	173	(100.0)	584 (63.3)	3.4	167 (45.1)	203 (54.9)	40
France	7 022	(92.4)	34 103 (57.5)	4.9	14 288 (55.4)	11 500 (44.6)	—
Greece	10	(66.7)	72 (69.9)	7.2	27 (57.4)	20 (42.6)	25
Ireland	776	(95.7)	4 978 (67.4)	6.4	1 582 (46.7)	1 804 (53.3)	39
Italy	474	(?)	3 297 (?)	7.0	2 066 (62.7)	1 281 (37.3)	450
Norway	139	(95.9)	801 (62.9)	5.8	282 (59.9)	189 (40.1)	—
Netherlands	1 728	(?)	9 485 (?)	5.5	4 417 (44.0)	5 628 (56.0)	2 872
Poland	556	(79.9)	2 156 (53.3)	3.9	1 841 (83.5)	365 (16.5)	4
United Kingdom	2 558	(?)	11 317 (?)	4.4	3 685 (51.0)	3 546 (49.0)	—
Czechoslovakia	5 349	(87.4)	25 037 (58.7)	4.7	19 317 (89.1)	2 365 (10.9)	1 397
Totals	25 858	(90.5)*	132 222 (60.0)*	5.1	71 379 (67.2)	34 832 (32.8)	5 164
							25 277

* For those countries supplying data.
(?) Percentages not specified.

Source: European Embryo Transfer Association 1990.

The extent to which ET is used in domestic animals depends entirely on economic factors, and therefore fluctuates with the livestock market. Competition also governs the costs and prices of transfers and there are many different ways in which services are offered to producers. Many practitioners charge the client by the hour (\$150 to \$200) and for the materials (~\$50) and hormones (~\$120) used, with a reduction in charges when several procedures are performed on the same premises. Generally, a dairy farmer can expect to pay ~\$300 to \$400 to have embryos recovered from a superovulated donor, and ~\$50 to \$100 to have each embryo transferred to his or her own recipient. If ET is to be used on a scale similar to AI, such costs and charges will have to be reduced.

New developments in artificial breeding, notably those involved in embryo manipulation and transfer, are, in some quarters, meeting the same kind of resistance and hostility that greeted AI nearly 50 years ago (Hammond 1962). This, undoubtedly, is largely because of both real and perceived implications for human reproduction and all that that means in our society. Understanding and judging the validity of such perceptions can be helped by a basic knowledge of reproductive biology and of similarities and differences between domestic animals and humans. This will be dealt with, briefly and selectively, in the following section.

Comparative Aspects of Reproductive Physiology

Although the basic mechanisms of reproduction are common to all eutherian mammals, there are major species-specific differences in most of the reproductive processes.

The Female Reproductive Cycle

Of most basic interest to the understanding of mammalian reproduction is the concept of the female reproductive cycle. As in women, females of all domestic species can have regular cycles regulated by the development of one or more ovulatory follicles during a follicular phase, ovulation and development of the corpus luteum to begin a progesterone-dominated luteal phase, and demise of the corpus luteum to initiate resumption of the follicular phase. However, the conventional definitions of the beginning of the cycle are based on externally obvious signs that differ between humans and domestic animals. In humans, the demise of the corpus luteum is followed by menstruation; the menstrual cycle, by definition, begins at the onset of menses. Conversely, menstruation does not occur in domestic animals, but the latter part of their follicular phase is marked by a period of overt sexual receptivity, known as estrus. Its onset defines the beginning of the estrous cycle. Consequently, whereas ovulation occurs in mid-cycle in the human, it occurs at the beginning of the cycle in domestic animals. Similarly, pregnancy is dated from the onset

of the last menses in humans, approximately 14 days before ovulation, but it is dated from estrus or the time of insemination in domestic animals, roughly coincident with ovulation.

As in women, the proximate control mechanisms of cyclicity in domestic animals rely on hormonal interactions among the hypothalamus, anterior pituitary, ovary, and uterus. However, in many domestic species, external factors play a major role in the control of the estrous cycle. In seasonal breeders such as the mare and ewe, ovarian cyclicity is limited to a specific period of the year, mediated through photoperiodic stimulation or inhibition of the hypothalamus and pineal gland. In general, the breeding season occurs at a time that will ensure that the young will be born in the spring. Consequently, in horses, having an 11-month gestation period, the breeding season occurs in spring, while in sheep, having a five-month gestation period, breeding occurs in late autumn. Male pheromones can hasten the onset of puberty and synchronize cyclicity in groups of ewes and sows. Similar olfactory stimuli are thought to be responsible for the synchronization of menstrual cycles among groups of women in communal housing such as boarding school dormitories. Lactation is a major regulator of cyclicity in domestic animals and in most large mammals, including humans. The exact mechanisms of lactational anestrus are not known but may involve a combination of visual and olfactory cues, and the mechanical stimulus of suckling. However, the resumption of cyclicity is closely associated with the intensity of suckling; in cows that are allowed to suckle their calves only intermittently, post-partum anestrus is significantly shorter than in cows continually exposed to their calves. Cyclicity is inhibited by poor nutrition or other stresses, possibly through the actions of endorphins or adrenal cortical hormones on the hypothalamus. Fertility is also reduced by very high energy diets.

As with the menstrual cycle, the length of the estrous cycle is largely determined by the life span of the corpus luteum. This differs among species, and so the estrous cycle is about 16 to 17 days in sheep and 20 to 21 days in goats, pigs, cattle, and horses. In humans, the regression of the corpus luteum (luteolysis) is induced by local ovarian estrogens and prostaglandins (PG). In domestic animals, luteal regression depends on one of these, PGF_{2 α} , produced by the uterus. Transport of PGF_{2 α} to the ovary is either directly from the uterine vein to the ovarian artery, as in cattle and sheep, or via the general circulation, as in horses and pigs. In cattle and sheep, at least, PGF_{2 α} causes the release of oxytocin from the corpus luteum, which induces further PGF_{2 α} release from the uterus, forming a positive feedback loop until the corpus luteum has regressed completely.

In cattle and sheep, two or three waves of follicular development occur during each estrous cycle, each wave producing a dominant follicle. The dominant and subordinate follicles in the waves produced during the immediate post-ovulatory period and in the mid-luteal phase normally regress; only the dominant follicle arising from the wave in the follicular

phase goes on to ovulate. However, given exogenous gonadotropin support and artificial induction of luteolysis, the follicles produced earlier in the cycle can continue to develop, ovulate, and produce fertile oocytes. This contrasts with what occurs in humans and pigs in which only a single wave of follicular development occurs, during the follicular phase proper (Driancourt 1991).

Fertilization and Early Embryonic Development

Only recently have we come to appreciate that remarkable interactions between the gametes (germ cells) and somatic (body) cells begin even before they unite to form the zygote. Thus, factors from the egg itself are involved in causing surrounding cells of the cumulus oophorus to expand in preparation for fertilization (Vanderhyden et al. 1990), and contact between spermatozoa and the epithelial cells lining the oviduct seems vital to preparing, selecting, or both, the survivor destined to pass its genes to the next generation (Pollard et al. 1991; Hunter et al. 1991).

After fertilization of the oocyte in the ampullary-isthmic region of the oviduct (fallopian tube), the interactions continue and multiply. The importance of these interactions is extraordinarily evident in the horse, in which eggs generally fail to pass through the oviduct to the uterus unless they are fertilized and cleave several times (Betteridge et al. 1979). This early autonomy of the horse embryo may involve very precocious production of PGE₂ (Weber et al. 1991), and it is becoming increasingly clear that a range of embryonic products, probably varying from species to species, remain to be discovered and understood for their roles in governing the earliest hours and days of pregnancy.

Morphologically, the early stages of embryonic development are similar among domestic species and humans. These include successive cleavage up to the morula stage of approximately 8 to 32 cells (blastomeres), at which stage aggregation and compaction of the blastomeres and formation of tight junctions lead to cavitation, differentiation between the trophoblast and inner cell mass, and hatching from the zona pellucida at the blastocyst stage. However, at this point, embryonic development of the domestic species diverges markedly from that in the human. Whereas the human blastocyst implants in the endometrium, the blastocysts of domestic species remain free within the uterine lumen and continue to grow through an extended pre-attachment period. For example, in ruminants, the blastocyst remains spherical to ovoid and grows for several days before beginning to elongate into a filament, reaching a length of five centimetres or more at day 16 of gestation in the cow (Betteridge and Fléchon 1988). It is only after this extensive elongation that the attachment of the embryonic vesicle to the endometrium begins, starting in the region of the embryonic disc and gradually extending out to the extremities of the vesicle. Development and attachment of the pig embryo are similar. In contrast, the horse conceptus remains spherical and migrates throughout

the uterus until it becomes fixed in one position at about day 16. Even then, the trophoblast has no direct contact with the endometrium for a further week because, in the horse, an acellular mucin-like glycoprotein capsule replaces the zona pellucida and completely envelops the conceptus until about day 22 (Betteridge 1989a; Betteridge et al. 1982; Enders and Liu 1991).

The genetic control of all the embryonic activities necessary to the initiation of pregnancy is at first maternal, a function of the messenger ribonucleic acid (mRNA) inherited through the oocyte. Soon, however, the embryo's own genome becomes activated so that its own genes control its function. This "maternal-zygotic transition" (MZT) is a pivotal event and one that seems to be especially vulnerable to adverse influences such as *in vitro* culture. In embryos of domestic species and humans, the MZT occurs at the 4-cell to 16-cell stage and they are, therefore, much closer to each other than to the mouse, where it occurs at the 2-cell stage (Telford et al. 1990). This makes farm animals much better models of this aspect of human development than the frequently used mouse. Another vital genetic event in the early embryo is the inactivation of one of the X chromosomes in females, so that double dosage of X-linked genes is avoided in the fetus and adult. Again, there are species variations in the timing of this event. Although detailed study of X-inactivation in embryos of domestic animals is only just beginning (e.g., Romagnano et al. 1987, for the horse; de la Fuente and King, unpubl., for the cow),¹ these studies are highly likely to furnish valuable models for advancing our understanding of analogous events (and their relevance to X-linked diseases) in humans.

The fact that the pre-attachment phase in domestic animals is so much longer than the preimplantation phase in humans has some important and practical implications: the period during which embryos can be transferred is correspondingly longer. Calves, for example, have resulted from the transfer of embryos up to 15 to 16 days after ovulation, by which time human embryos would be firmly implanted. This gives access to much more advanced animal embryos for manipulation; as described later, the first sexed calves were produced by taking pieces of trophoblast from such elongated embryos (without affecting the embryonic disc) for cytogenetic analysis. It is not impossible that manipulations of the embryonic disc itself will become feasible in ruminants but will remain quite impossible in human embryos because of their inaccessible position in the uterus. The long pre-attachment phase also makes it possible to study trophoblastic function more closely with a view to making use of the cells themselves, or their products, for increasing the survival of transferred embryos in domestic animals (see below).

The establishment of the placenta in the domestic species is best described in terms of "attachment," in contrast to the process of "implantation" found in higher primates and rodents (Flood 1991). Both involve further interactions between fetal and maternal tissues that are very complex and change continuously. The form and function of the placenta

also vary widely among the domestic species and humans. A useful functional classification is according to the number of layers that separate fetal and maternal circulations. Other classifications take account of the shape of the placenta, or whether maternal tissue is lost, or fetal tissue retained, at parturition (*ibid.*). These variations are extremely important in considering the new reproductive technologies, for three major reasons. First, embryonic development and uterine development have to be "in step" (synchronized) if placentation is to ensue. Second (as already mentioned), the window of time during which ET is possible is generally longer in domestic animals than in humans. Third, the success of attempts to transfer embryos between different animal species depends on whether uterine and embryonic changes are sufficiently compatible to allow placentation.

Maternal Recognition of Pregnancy

In the wild, sexually mature females are expected to be either pregnant or lactating or both. The estrous cycles of animals have, therefore, probably largely evolved as mechanisms by which females failing to become, or to remain, pregnant return to breeding condition with minimal delay. Only in domesticated animals with contrived mating systems are repeated estrous cycles considered to be "normal." One of the first critical events after fertilization, therefore, is abrogation of the "fail-safe" mechanisms that bring about a return to estrus in the absence of pregnancy. What has to be accomplished is maintenance of the progesterone production that is a *sine qua non* of pregnancy in mammals. Progesterone in early pregnancy originates from the corpus luteum that forms within the ovarian follicle vacated by the egg, and so maintenance of pregnancy can be equated with maintenance of the corpus luteum. It is fundamental to appreciate that the developing embryo plays an active role in this process — it is by no means just a passive passenger in the uterus — although the interactions between the embryo and the mother to effect it are usually referred to as the "maternal recognition of pregnancy."

The mechanisms of maternal recognition of pregnancy that lead to the maintenance of the corpus luteum of pregnancy differ among the domestic species and humans. The concepti of domestic animals produce no known functional correlate to the human chorionic gonadotropin (hCG), which is produced by the human conceptus soon after implantation. Furthermore, whereas hCG has a direct stimulatory (luteotrophic) effect on the corpus luteum, cattle and sheep embryos produce trophoblastic proteins that inhibit uterine PG production, release an indirect anti-luteolytic mechanism, or both. These proteins include placental lactogens, pregnancy-associated glycoproteins, and a family of interferon-like compounds. Sulphated estrogens produced by the pig conceptus may be both luteotrophic and anti-luteolytic, and a minimum of four living concepti is required to maintain pregnancy. Cells from the embryonic girdle of the

horse conceptus migrate into the maternal endometrium and secrete equine chorionic gonadotropin (eCG or PMSG) from about 34 to 140 days of pregnancy. Although eCG is very potent when administered to other species, its exact function in the mare is unclear and controversial. Its ability to induce auxiliary corpora lutea in the pregnant mare's ovaries may be important (Murphy and Martinuk 1991).

Gestation and Parturition

The gestation period in domestic animals is approximately proportional to the size of the adult animal, varying from 113 days in the pig to 350 days in the horse. Unlike the human, where sufficient progesterone is produced by the placenta to maintain pregnancy from the sixth week onward, the corpus luteum is an obligate source of progesterone for the first half of pregnancy in the sheep, horse, and cow, and throughout pregnancy in the goat and pig. In the horse, the fetal-placental unit also secretes large quantities of B-ring unsaturated estrogens (equilin and equilenin) in the latter half of gestation.

The mechanisms responsible for the initiation of parturition have been best described in the sheep and goat, where the initial trigger is the maturation of the fetal pituitary-adrenal axis, resulting in an increase in fetal cortisol production. For these and other species, including the human, subsequent steps involve steroid hormones, PGs, relaxin, and oxytocin, all interacting to increase uterine contractility and cause expulsion of the fetus. In the present context, the active role of the term fetus in this process must again be emphasized, underlining the necessity for compatibility between the transferred embryo and the recipient, especially in inter-species transfers.

Even this cursory look at the physiological processes that are involved in initiating and maintaining pregnancy should help us to understand that relatively precise synchrony between the development of the embryo and of its maternal environment is required. The mechanisms bringing about such synchrony have evolved over the millennia, and perhaps we should not be so surprised that emulating them during the transfer of an embryo from one individual to another is difficult and not always successful. With this in mind, let us consider the steps involved in ET in some sort of chronological order. In doing so, we shall draw heavily on another recent summary of the subject (Betteridge 1993).

Current Procedures in Embryo Transfer

Selection of Donor and Recipient Animals for Embryo Transfer

The selection of a particular animal as a donor for ET depends on the objectives of the program. Additionally, procedural factors must be

considered in making selections because of their effects on the results that are likely to be achieved. Reproductive soundness is one such factor, and a thorough veterinary examination for any anatomical or pathological feature that would prevent an animal from conceiving is an advisable first step. Regular cyclicity is another major criterion for selection. In cattle, for example, it is recommended that cows undergo at least three normal cycles after calving before being used. Fat animals are to be avoided because of the difficulties of manipulating the reproductive tract and because overfeeding is often associated with reproductive dysfunction. Depending on the application, donors may be used as heifers or cows and either repeatedly or intermittently between calvings. These factors affect the ease of embryo collection (heifers being more difficult) and response rates (cows that have calved three or four times making the best donors) (Greve and Callesen 1989).

Recipients must also be reproductively sound and mature enough to carry, and give birth to, calves of the donor breed. Again, there is good evidence that an adequate diet is required to maintain fertility. However, in sheep, high levels of nutrition have a detrimental effect on pregnancy rates in normally mated (not recipient) animals (Parr et al. 1986).

Synchronization of Estrous Cycles

It is often necessary to synchronize the estrous cycles of groups of animals, for two reasons. First, significant fixed costs are associated with the assembly of materials and personnel required for procedures such as AI and ET; consequently, it is much more economical to perform such procedures on synchronized groups of animals at one time. Second, for direct ET, the estrous cycle of the recipient animal must be in synchrony with the developmental stage of the embryo and, therefore, with the estrous cycle of the donor. For ET in large-scale operations, natural synchrony can be used by observing large numbers of potential recipients for estrous behaviour once or twice a day. However, a continuously replenished herd of over 400 cyclic heifers or cows is needed to provide 20 recipients per day. Consequently, estrous synchronization is generally used to reduce the enormous expense of maintaining such large herds.

Two approaches can be used, alone or in combination, to synchronize estrus: induction of luteolysis with PGF_{2α} or its analogues to shorten the luteal phase, and treatment with progestagens in intravaginal devices or subcutaneous implants to artificially extend the luteal phase. The most important peculiarity of their use in ET is that donors stimulated with gonadotropins to induce superovulation come into estrus more rapidly after luteolysis (or withdrawal of exogenous progestagens) than do most unstimulated recipients. Therefore, to synchronize estrus with PGs, for example, recipients need to be treated 12 to 24 hours before the donors.

Similar principles apply to the selection and synchronization of donors and recipients in sheep and goats, but the situation can be complicated by

the effects of seasonal breeding in these species, as is discussed below in relation to superovulation.

In pigs, synchronization of estrous cycles is made difficult because of the prolonged insensitivity of the corpora lutea to PG-induced luteolysis. Various schemes have been devised to overcome this. One method is to use PG to induce early abortion and synchronized returns to estrus in bred gilts. Another is to feed progestagens for six days in the latter half of the luteal phase of cyclic gilts so that their own corpora lutea regress during the feeding period and synchronized returns to estrus follow withdrawal of the progestagen. However, in sows, probably the most widely used method of synchronization is weaning, which results in estrus four to seven days later (Pope 1989).

In horses, selection of donors can be of special concern because, even in the breed societies that accept ET, some degree of infertility often needs to be proved before a mare can be used as a donor. This, and the fact that superovulation cannot be induced easily in mares, can seriously compromise the success rates obtained, and so it is important to diagnose the reasons for a mare's infertility before deciding whether ET is warranted. Synchronization of donors and recipients poses an additional problem in horses because of variation in the length of estrus and the time of ovulation after the onset of estrus. However, follicular development can be closely monitored by transrectal palpation or with the aid of ultrasound. Follicles attaining a certain diameter can then be induced to ovulate by injecting the mare with hCG, making it possible to achieve good results in reproductively healthy mares by repeatedly collecting and transferring single embryos from donors kept in approximate synchrony with two to four potential recipients by repeated use of PGs (Sirois et al. 1987). Of special interest in horses is that ovariectomized mares treated with progesterone by injection, or orally active progestagens, can be used as recipients. Also, this progesterone treatment scheme is convenient because it need not be initiated until ovulation has been diagnosed in the donor (Hinrichs et al. 1985; Squires 1989; Squires et al. 1989).

Obtaining Embryos by Superovulation

The basic physiological principle for superovulation (ovarian stimulation) is common to humans and domestic animals: increased circulating follicle-stimulating hormone (FSH) supports the development of subordinate follicles that would normally succumb to atresia (see Moor et al. 1984). Three approaches to increasing the amounts of circulating FSH are: to treat with exogenous FSH or FSH-like hormones (Monniaux et al. 1983); to inhibit the natural feedback inhibition of estradiol on the hypothalamus with weak estrogen agonists or antagonists such as clomiphene citrate and thus increase gonadotropin-releasing hormone (Gn-RH) and gonadotropin secretion (Huppert 1979); or to administer pulsatile Gn-RH to stimulate gonadotropin secretion directly (Filicori et al. 1991). In

domestic animals, treatment with exogenous FSH (or FSH-like hormones) has been, and continues to be, the most usual approach (see Betteridge and Smith 1988; Mapletoft et al. 1991; Boland et al. 1991), although it has been shown that Gn-RH can also induce follicular development and ovulation in post-partum beef cows (Walters et al. 1982) and seasonally anestrous ewes (McNeilly et al. 1982), goats (Knight et al. 1988), mares (Ainsworth and Hyland 1991), and deer (McLeod et al. 1991).

The gonadotropins used for superovulating domestic animals are derived from several sources (see Boland et al. 1991). Originally, eCG, which possesses both FSH and luteinizing hormone (LH) activity, was commonly used. In fact, it is said that more donors have been treated with one particular batch of eCG from Argentina (at Alta Genetics, Calgary) than any other single preparation to date. However, in North America at least, eCG has been largely supplanted by semi-purified preparations of FSH (with varying amounts of LH) extracted from the pituitaries of slaughtered animals, usually pigs. Most recently, bovine FSH has been produced by recombinant deoxyribonucleic acid (DNA) techniques with encouraging results (Wilson et al. 1989). There is general agreement that any of these gonadotropins has to be injected during the mid-luteal phase of the donor's estrous cycle in such a way as to maintain high circulating levels for about four days. Luteolysis is initiated by one or two injections of PG, usually on the third day of exposure to gonadotropins. The high levels of gonadotropins are maintained naturally and conveniently after a single injection of eCG because it is highly glycosylated and therefore has a long half-life in the circulation. FSH, on the other hand, has a much shorter half-life and is therefore usually injected twice daily, which is much less convenient. However, a single subcutaneous injection has now been shown to be effective (Hockley et al. 1992). Despite the inconvenience of using multiple injections of FSH, its use has become standard throughout North America since its apparent superiority over eCG was rediscovered in the mid-1970s. In goats (Armstrong et al. 1983) and sheep (L.C. Smith 1988), the evidence for better responses to FSH than to eCG is convincing. However, in Europe, eCG continues to be used quite extensively, often in combination with antisera that are used to remove from the circulation any eCG that persists after the treated donor has exhibited estrus (Dieleman et al. 1989). The rationale is that continued gonadotropic stimulation after ovulation will induce another, unwanted, crop of follicles that produce estrogens, upset the "normal" endocrine milieu, and adversely affect the quality of embryos developing from the primary crop. However, whether the antisera significantly improve results remains controversial (Greve and Callesen 1989).

The major limitation to superovulation in cattle is the unpredictability and variability in both the total numbers and viability of embryos resulting from a given treatment (Betteridge 1977; Greve and Callesen 1989; Boland et al. 1991). Variability in the biological activity of the preparations of

gonadotropins used to induce superovulation is probably a major source of the variability in the ovulatory response. In the case of eCG, both the degree of glycosylation and the ratio of FSH to LH activity vary significantly between mares, and over the gestation period within mares (Murphy and Martinuk 1991). Consequently, the biological activity of commercial preparations of eCG can vary significantly from batch to batch, depending on the population of mares from which it was derived. Pituitary preparations of FSH contain varying amounts of LH, and the superovulatory response in cattle decreases with the ratio of FSH to LH (Murphy et al. 1984). Some controversy surrounds the FSH/LH ratio that should be used in purified preparations of pituitary gonadotropins. A contributing factor to the uncertainty is probably the fact that the assays of FSH and LH activities (both biological and immunological) may not relate very closely to the physiological effects required of the hormones.

The variability in the ovulatory response to exogenous gonadotropins is also probably related to the facts that the gonadotropin treatments are (a) superimposed on the treated animal's endogenous endocrine system and (b) affect only one component of the system that normally leads to the production of a fertile ovum. Consequently, research is in progress that aims to reduce the variability that may be due to endogenous hormones, and to take account of hormones, growth factors, and substances other than the gonadotropins that may influence embryo quality.

One approach is to attempt to increase the number of subordinate antral follicles that can be rescued from atresia and thus increase the ovulatory response to superovulation (Monniaux et al. 1983). A "priming" injection of FSH given early in the cycle, approximately seven days before the initiation of superovulatory FSH treatment, has been reported to result in increased ovulatory response in cattle in some cases, but not others, suggesting that such priming is advantageous only in cows that have an otherwise poor response to superovulatory stimulation (Rieger et al. 1988; Boland et al. 1991). A recent report has suggested that such priming may double the ovulation rate in mares (Sirois et al. 1992).

Although gonadotropins are the primary stimulators of follicular development and function, it is now apparent that intrafollicular growth factors, particularly insulin-like growth factor (IGF-1), act to potentiate or fine tune the follicular mechanisms (Dorrington et al. 1987; Hammond et al. 1991). Growth hormone (somatotropin) stimulates the production of IGF-1 in various tissues, and several clinical experiments have demonstrated that the follicular growth response to human menopausal gonadotropin (hMG) in women is related to the potential for endogenous growth hormone secretion (Menashe et al. 1990), and can be facilitated by co-treatment with recombinant human somatotropin (Blumenfeld and Lunenfeld 1989; Homburg et al. 1990; Ibrahim et al. 1991). Treatment with growth hormone or immunization against somatostatin has similarly been shown to increase the ovulation rate in pigs (Kirkwood et al. 1988), sheep (Kirkwood et al. 1990), and superovulated cattle (Herrler et al. 1990; Rieger

et al. 1991a). However, longer-term growth hormone treatment may produce a much more dramatic effect. Gong et al. (1991a, 1991b) have shown that treatment of heifers with recombinant bovine growth hormone over two estrous cycles doubles the number of small to mid-size antral follicles. The effect of such long-term treatment on the ovulatory response to superovulatory treatment has not yet been tested, but it has much promise, because it is the small to mid-size antral follicles that are rescued from atresia by superovulatory regimens. This approach could be of significant benefit in human medicine, and the cow provides an excellent model in which to study that possibility.

The effect of the dominant follicle on the response to superovulatory treatment in cattle is also interesting. Guilbault et al. (1991) have demonstrated that the ovulatory response to superovulatory treatment is markedly reduced when a functionally dominant follicle is present (see also Roche and Boland 1991). This effect is clearly not simply due to negative feedback of inhibin or ovarian steroids on the hypothalamus or anterior pituitary at the time of superovulation, because any such effect on endogenous gonadotropin secretion would be overwhelmed by the exogenous gonadotropins. The exact mechanism involved has yet to be elucidated, but is probably systemic rather than purely local. Consequently, it may well be possible to circumvent this inhibitory effect and improve the ovulatory response, in humans as well as in domestic animals.

The efficacy of superovulation is also limited by the viability of the embryos produced. Whereas 80 percent or more of embryos produced in unstimulated cattle are morphologically normal when collected at day 7 of gestation (Ayalon 1978; Maurer and Chenault 1983), only 65 percent of embryos collected after superovulation are morphologically normal, and, of those, only 60 to 80 percent result in pregnancy when transferred to recipient animals (Table 2). Several factors might be responsible for this phenomenon, including unsuitability of the recipient uterine environment, but much of it is probably due to the developmental incompetence of many of the embryos. As discussed later, significant economic gains could be realized by a reliable *in vitro* test of potential viability, but approaches to increasing the proportion of viable embryos produced by superovulation are of equal, or perhaps greater, interest.

One such approach is suggested by the observation that, because of the more rapid rise in circulating estradiol, the pre-ovulatory LH surge and ovulation occur approximately 24 hours earlier in superovulated animals than in unstimulated animals, relative to the time of luteolysis. Consequently, a significant proportion of the oocytes are functionally immature when ovulated and so either cannot be fertilized or, if fertilized, develop abnormally. In women undergoing ovarian stimulation for IVF, it is now common practice to address this problem by medium-term treatment with Gn-RH agonists (Gn-RHa) to down-regulate the pituitary. However, such treatment requires Gn-RHa administration by multiple injections

or infusion and also often produces an initial undesirable stimulation of endogenous gonadotropin secretion and follicular development (flare-up) (Loumaye 1990). Similar results have been obtained with Gn-RHa treatment of sheep (McNeilly and Fraser 1987). Conversely, Gn-RH antagonists, given only during the follicular phase of stimulated cycles, require much shorter-term treatment and do not produce ovarian flare-up (Frydman et al. 1991). Only limited attention has been given to this approach in women, but, in cattle, it has been shown that a 60-hour treatment with a potent Gn-RH antagonist can delay the LH surge and ovulation in superovulated heifers for 24 hours, to approximately their times of occurrence in unstimulated animals (Madill 1992).

Table 2. Pregnancy Rates Obtained with Embryos Classified Morphologically into Three or Four Grades of Descending Quality in Five Separate Studies

Morpho-logical grade*	Pregnancy rate (%)				
	Elsden et al. 1978	Shea 1981	Lindner and Wright 1983	Hasler et al. 1987 (up to 1983)	Hasler et al. 1987 (from 1985)
A	173/275 (63)	5/7 (71)	130/292 (45)	4 037/5 521 (73)	451/542 (83)
B	88/152 (58)	576/672 (56)	128/292 (44)	181/304 (60)	307/408 (75)
C	13/42 (31)	57/130 (44)	40/149 (27)	31/76 (41)	135/214 (63)
D	5/42 (12)		10/50 (20)		6/13 (46)

* Classifications vary from author to author; in this table, grade A is best, grade D is worst.

As previously noted, the mare appears to be particularly resistant to superovulatory treatment. A novel and ingenious approach to overcoming this limitation has been developed by Hinrichs and DiGiorgio (1991). Ova are collected from ovaries of other mares and transferred into the dominant follicle of the recipient animal where they undergo maturation and are ovulated with the native ovum in response to the endogenous LH surge. Though of considerable experimental interest, there are obviously limits to the clinical usefulness of this approach when the genetic origin of the oocytes is critical.

Some attention must also be paid to fertilization of the ova produced by unstimulated or superovulated donors, which is accomplished by normal mating or, much more commonly, AI with semen from a selected sire. In cattle, it has become usual to inseminate prospective donors a second time about 12 hours after the initial insemination. The first insemination is

timed in the usual way to be in the afternoon in animals first seen exhibiting estrus in the morning, or on the morning after an estrus is first detected in the afternoon, in either case no later than 60 hours after PG. The rationale for repeating the insemination is that multiple ovulations occur over a span of time and so a supply of viable spermatozoa must be made available for a correspondingly longer time than in single-ovulating animals. However, semen quality of individual bulls is probably the main factor governing results, and it is by no means certain that multiple inseminations are necessary (Greve and Callesen 1989). Mares and sows are inseminated, naturally or artificially, as usual.

In sheep, superovulation (particularly when induced with eCG or when they are out of season) is followed by low rates of fertilization unless special attention is paid to insemination (Armstrong and Evans 1984; Tervit 1989). Superovulated ewes need to be run with rams at a ratio of no more than six ewes per ram because the reduction in fertility after induction of superovulation outside the normal breeding season may be due more to effects on the ram than to effects on the ewe. Intrauterine AI, performed laparoscopically or through a minor abdominal incision under local anaesthesia, is another way of improving results, particularly if frozen semen is to be used. Newly developed transcervical insemination techniques may obviate the need for even these surgical interventions (Buckrell et al. 1992). There is some evidence that the incorporation of Gn-RH into the superovulation regimen can improve fertilization rates achieved with natural and laparoscopic insemination (Walker et al. 1989). Out-of-season superovulation can be achieved in goats treated with progestagens, with or without PGs, followed by gonadotropins. Embryo yield may be reduced by seasonal effects unless does are hand-mated twice during estrus. Oocyte quality may also be affected by season (Baril and Vallet 1990).

Collecting Embryos

Until the late 1970s, embryos were collected from the uteri of superovulated cattle by surgery under general or local anaesthesia (see Betteridge 1977). However, transcervical methods are now universal and all depend upon the introduction of controlled quantities of a flushing medium into the uterus through a cuffed catheter, recovering it into a container, and searching the recovered fluid for the embryos that it has flushed from the uterus. The medium is usually a phosphate-buffered saline supplemented with heat-treated serum and antibiotics. It may be used as repeated injections followed by re-aspiration of small volumes (~60 mL) with a syringe, or as aliquants introduced by gravity flow that is interrupted to allow recovery when the operator feels the uterine horn or horns to be sufficiently distended. The collected flushes are normally filtered to speed embryo recovery by reducing the volume of fluid that needs to be searched under the stereomicroscope. The filters may be in-line

during recovery or used to filter the combined flushes when they are all recovered.

Mares are flushed by analogous techniques that are made easier by the distensibility of the equine cervix. Pigs and the small ruminants are virtually always flushed surgically, and techniques have changed little over the years (Betteridge 1977), except that laparoscopic recovery from sheep and goats is possible in skilled hands (McKelvey et al. 1986).

The timing of recovery is dictated by location of embryos in the reproductive tract in given species at various times after ovulation (Betteridge 1977; Betteridge and Fléchon 1988; Betteridge et al. 1989a). In cattle, for example, most embryos remain in the oviduct until day 5. However, delaying recovery beyond day 5 reduces the chances of obtaining precompaction morulae that are required for some kinds of micromanipulation. For routine ETs and cryopreservation, cattle embryos are usually collected on day 7, as morulae and blastocysts.

In mares, timings are related to the day of ovulation (day 0) rather than estrus. The embryo does not enter the uterus until day 5.5 to 6.0, and recovery rates are usually better on day 7 than earlier. However, delay beyond day 8 is inadvisable for routine transfer because older equine embryos produce lower pregnancy rates when transferred and tolerate cryopreservation less well than younger ones (Squires et al. 1989; Rieger et al. 1991b).

Sheep, goat, and pig embryos can be as easily collected from the oviduct as from the uterus at surgery. Choice of the time of collection, therefore, depends on the stage of embryonic development required for any particular manipulation and for producing optimal pregnancy rates when transferred. The usual day for collection and transfer is day 6 to 7 in sheep. Superovulated goats are often flushed before day 5, to avoid the depletion in embryo yield that occurs in the considerable proportion of animals in which corpora lutea regress prematurely, particularly in the fibre-producing breeds (Armstrong et al. 1983). Premature regression of corpora lutea in superovulated goats seems to vary with the gonadotropin used (McNatty et al. 1989) as well as the breed. It may be reduced by treatment of donors with intravaginal progesterone (Gilbert et al. 1990). In pigs, most routine transfers are performed on or before day 6; embryos are collected from the oviducts alone up to day 3, but progressively longer portions of the uterus need to be flushed as pregnancy advances (Pope 1989).

Assessing the Quality of Embryos

Considerable costs are involved in the preparation and maintenance of recipient animals for ET, particularly in cattle. Consequently, much effort has been directed toward developing methods for assessing the potential viability of embryos to ensure the maximum probability of successful pregnancy. In practice, the assessment is based on visual

appraisal of the embryo's morphological structure using a stereomicroscope or inverted compound microscope. Such subjective assessment demands familiarity with normal embryo development and considerable experience. Most morphological classification systems are based on criteria such as overall shape, uniformity of blastomeres, cell density, vacuolization, fragmentation, and developmental retardation (Elsden et al. 1978; Shea 1981; Lindner and Wright 1983). In these schemes, embryos are typically assigned morphological scores in four or five classes ranging from "excellent" to "degenerate," "unfertilized," or both. When practised uniformly by skilled observers, such a classification does bear a useful relationship to the results of transfer of populations of embryos, in that a larger proportion of morphologically "excellent" embryos produce pregnancies than do morphologically "poor" embryos (Table 2). However, predicting the viability of an individual embryo is much less reliable. Consequently, deciding whether or not to transfer a particular embryo involves consideration of several other factors, including the availability of suitable recipients and the potential value of a calf from a particular donor.

Numerous attempts have been made to make morphological evaluation of embryos more objective (Betteridge et al. 1989a; Butler and Biggers 1989; Meinecke and Meinecke-Tillman 1990). Most make use of vital dyes that affect cells differentially, depending on the intactness of cell membranes and, in some cases, the activity of intracellular enzymes. In dye exclusion tests, the principle is that the intact cell membrane prevents the stain from permeating the cell so that live cells do not stain whereas dead ones do. In dye incorporation tests, on the other hand, the live cells stain (fluoresce) because their intracellular enzymes break down a dye (e.g., fluorescein diacetate) to form a fluorescent product that is then retained by their intact cell membranes. Neither these dye tests, nor more elaborate tests made on the basis of immunological detection of cell surface antigens or image analysis, have yet proved to be better predictors of viability than visual assessment, and they have not become widely used.

Early embryo development requires the production and expenditure of large amounts of cellular energy for cell growth, division, and differentiation (Rieger 1992); therefore, non-invasive measures of energy metabolism may be better indicators of potential viability than membrane integrity (Rieger 1984). A direct relationship between glucose uptake and subsequent viability was first demonstrated in Day-10 to -11 cattle blastocysts (Renard et al. 1980), and subsequently confirmed in mouse blastocysts (Gardner and Leese 1987). An intermediate level of pyruvate uptake has been shown to be related to the subsequent viability of human embryos (Turner et al. 1991). Oxygen uptake has been shown to be related to the viability of mouse embryos (Overstrom et al. 1989), and this relationship is under investigation in cattle embryos (Overstrom et al. 1992).

Little success has been achieved in relating specific embryo products to viability except that the production of plasminogen activating factor by embryos may be related to their subsequent development, at least *in vitro*

(Kaaekuahiwi and Menino 1990). Attempts to use production of immunosuppressive activity and platelet activating factor for this purpose in cattle and sheep have been disappointing (Croy et al. 1988; Battye et al. 1991).

Short-Term Embryo Culture

Current practices in commercial ET, including cryopreservation, normally require short-term culture of embryos collected at the morula or blastocyst stage. The requirements for this relatively limited culture are substantially different from those for the longer-term culture of embryos produced by IVF, which are discussed later. However, in the numerous steps involved in the transfer of embryos between the uterus of the donor and the uterus of the recipient (Table 3; Rieger and Betteridge 1989), compromises are made between technical efficiency and physiological optima. Consequently, the embryo is exposed to a variety of environmental changes and stresses that would not occur *in vivo*, and considerable effort is required to limit or compensate for these stresses (Table 4; Rieger and Betteridge 1989).

Although it might be argued that the best way to maintain the embryo outside the female is to duplicate natural conditions *in vitro*, it would be highly impractical, if not impossible, to modify all of the procedures involved in commercial ET for that purpose. In addition, optimal maintenance of embryos *in vitro* could possibly be achieved under conditions very different from those pertaining *in vivo*. Whereas conditions within the oviduct would be expected to be relatively consistent for a given developmental stage, media of markedly different composition can support development of mouse embryos *in vitro* (Lawitts and Biggers 1991).

Table 3. The Procedures and Components of Embryo Transfer and the Associated Factors That Can Affect Embryos Between Collection and Transfer

Collection	catheters, tubing, flushing fluid, holding vessels, temperature, lighting
Isolation	filters, searching dishes, embryo-handling pipettes, holding medium
Culture	medium (water, salts/osmolarity, buffer/pH, energy substrates, vitamins, amino acids, serum/macromolecules, growth factors, antibiotics), atmosphere (CO ₂ , O ₂), volume, physical substratum, co-culture
Manipulation	cryopreservation, viability testing, bisection/biopsy, sexing
Transfer	medium, pipettes

Table 4. A Comparison of the *In Vivo* Environment of Preimplantation Embryos with *In Vitro* Culture Conditions

<i>In vivo</i>	<i>In vitro</i>
Constant temperature	Periods of exposure to ambient temperature and varying rates of temperature change
Total darkness	Periods of exposure to ambient light and to microscope lamps
Oxygen and carbon dioxide tensions controlled by uptake, diffusion, and production by maternal tissues	Direct exposure to atmospheric gas concentrations with little modification
Minute envelope of surrounding fluid	Comparatively large surrounding fluid volume
Continuous dynamic exchange of substrates and metabolites between the embryo and the maternal tissues	Intermittent replacement of medium. Slowly decreasing supply of substrates and dilution of metabolites

Of major concern are the effects of exposure to temperature and light on the embryos. It is, perhaps, not only the absolute differences in temperature encountered by the embryo that are important, but also the rates of change and the numbers of fluctuations involved. Experimental studies in rabbits indicate that exposure of embryos to room temperature and ambient light, especially in combination, severely inhibits cell division (Fischer et al. 1988). Bovine embryos may be relatively tolerant of repeated cycles of temperature change between 0°C and ambient temperature (Lehn-Jensen 1986), although the effects on development have not been examined in the same detail as in rabbit embryos.

Practical limitations require that the media used for collection and isolation of embryos be acid-base buffered with phosphate salts or Hepes, instead of bicarbonate. This is of considerable concern because bicarbonate-buffered media are usually considerably better for the development of embryos of all species, including those of domestic animals (see Wright and Bondioli 1981). However, it may be possible to minimize this problem by including low concentrations of sodium bicarbonate (Y. Ménézo, pers. comm., 1989) or permeant weak acids (Bavister 1988) in phosphate-buffered media.

Similarly, many studies have shown that atmospheric oxygen tension is detrimental to the development of embryos of several species, probably

due to the production of toxic oxygen radicals (see Rieger 1992). Although it would be difficult to eliminate exposure to the atmosphere throughout the procedures of commercial ET, the inclusion of reducing agents, such as ascorbic acid, α -tocopherol, or β -carotene, in the culture medium may be beneficial (Ménézo 1976).

Complex culture media that have been developed for somatic cells (e.g., Ham's F-10) or mouse embryos (e.g., M12) can support development of later-stage embryos from domestic animals through blastulation, expansion, and hatching to a reasonable degree. However, it is becoming increasingly apparent that the metabolic activity, and hence the nutritional requirements, of embryos of domestic species differ considerably from those of mouse embryos. In particular, whereas the energy requirements of the mouse blastocyst can be met by anaerobic metabolism alone (Leese 1990, 1991), recent studies have indicated that the blastulation, expansion, and hatching of sheep (Gardner and Batt 1991), cow (Tiffin et al. 1991), and horse (Rieger et al. 1991b) embryos require both anaerobic and aerobic metabolism. These results suggest that it may be necessary to increase the concentration of Krebs cycle substrates such as glutamine and pyruvate in media for the culture of embryos of domestic animals.

Using current culture techniques, cattle embryos can hatch from the zona pellucida, and continue to grow into spheres, approaching one millimetre in diameter (S.P. Leibo, pers. comm., 1992). However, we are unaware of any reports of successful culture to elongation, the subsequent morphologically significant developmental event.

Sanitation and Disease Control in Embryo Transfer

Embryos transferred between donor and recipient animals could be vectors for the transmission of contagious diseases. Consequently, the identification of potentially significant pathogens and the development of appropriate sanitation procedures for embryos are of major importance, particularly for the purposes of international export. The scientific background and recommendations for sanitary procedures for ET have been reviewed by Thibier (1990). In the Canadian context, it should be stressed that most of the relevant research in this area has been done by Agriculture Canada scientists at the Animal Diseases Research Institute in Ottawa (see Singh 1987a, 1987b; Hare et al. 1976; Stringfellow and Seidel 1990), a fact recognized by the Distinguished Service Award recently presented by the International Embryo Transfer Society to Dr. W.C.D. Hare.

The research committee of the International Embryo Transfer Society, in collaboration with l'Office international des épizooties, has identified approximately 50 different pathogens, including viruses, bacteria, fungi, and ureaplasmas, which are potentially of concern. Diseases that are already categorized as presenting negligible risk of transmission with bovine embryos are enzootic bovine leucosis, foot and mouth disease, and brucellosis, but each disease of potential risk has to be evaluated

separately. Moreover, extrapolation from one infectious agent to another within a livestock species, or from one livestock species to another for a given infectious agent, is not possible.

Six classes of risk of contamination have been recognized: intracellular contamination of gametes, contamination at the time of fertilization, contamination in the uterine environment, contamination in the process of collection, contamination *in vitro*, and contamination at the time of transfer. No evidence yet suggests that the first two classes are of concern, at least in domestic animals. For the other classes, all available evidence indicates that pathogens cannot penetrate an intact zona pellucida, although some viruses, mycoplasms, and ureaplasmas can bind to it.

The recommendations of the International Embryo Transfer Society (1989) are therefore that:

1. the embryo have an intact zona pellucida and be free of cellular debris; and
2. the embryo be passed through at least 10 baths of sterile culture medium containing antibiotics and, if required, trypsin (to remove adhering pathogens), using a fresh sterile pipette for each passage, ensuring that medium transferred from bath to bath with the embryo is diluted at least 100-fold at each passage.

The fact that the zona pellucida of each embryo must be intact to meet these requirements is, at present, an obstacle to the international movement of embryos that have been biopsied (e.g., for sexing, see below) or in which the zona pellucida has been inadvertently ruptured during cryopreservation (see below).

In addition to the requirements recommended by the International Embryo Transfer Society, specific regulations of importing countries may be applied. For example, France's Ministry of Agriculture requires that recipients of embryos imported from North America be held in quarantine (Thibier 1990).

The advent of *in vitro* production as a means of providing embryos has introduced new potential problems of disease transmission that are only just beginning to be addressed. From preliminary experimental work, the screening of media used for oocyte maturation might possibly be a way of monitoring for some viruses (Thibier 1990). Should this become a requirement for the use of embryos produced *in vitro*, it will become essential to cryopreserve them while the tests are completed — a challenge that is only just beginning to be met.

It is significant that even given the potential risk of disease transmission, Thibier (1990) suggested that ET may be the safest way of exchanging genetic material internationally, when compared with the trade in animals. It is also a unique way of eliminating disease by producing disease-free offspring from infected dams.

Although these considerations apply primarily to embryos moved geographically ("in space"), they are equally true of gametes and embryos moved "in time" by collection and cryopreservation at one time, then thawing and using them for breeding purposes later. In the human context, it is possible that people might want to cryopreserve their gametes or embryos, for example, before undergoing therapy for testicular cancer, or that patients with a vertically transmissible infectious disease might desire to use ET to produce genetically related healthy children.

Cryopreservation

Often, embryos must be preserved over prolonged periods between collection (or *in vitro* production) and transfer, for several reasons. First, a need often exists to transport the embryos over long distances, particularly for international trade. Second, there may be too few recipients at the required stage of their estrous cycles when the embryos are collected. Third, it may be desirable to store embryos pending the results of tests (e.g., health tests on the donor, sexing biopsies taken from the embryo, or performance tests on siblings). Fourth, stored embryos could be useful in genetic conservation programs, both for endangered species and for particular lines of animals. Fifth, recipient oocytes or parts of embryos (half-embryos or blastomeres) may need to be stored in preparation for nuclear transplantation (Westhusin et al. 1991), perhaps after the performance of other parts has been assessed. Consequently, there has been much incentive to develop methods of freezing and thawing (more correctly, cryopreserving) embryos.

Embryo cryopreservation in liquid nitrogen is a technique that is taken almost for granted today and yet was quite unheard of when the commercial practice of ET began. Great credit is due to a limited number of investigators who have used basic cell biology in developing practicable, efficient methods since the first successful cryopreservation of a mammalian embryo (Whittingham et al. 1972; Wilmut 1972). It is less than 20 years since the first domestic animal embryo survived freezing and thawing (Wilmut and Rowson 1973).

The basic principle of cryopreservation is that all biological and most chemical processes cease at the temperature of liquid nitrogen (-196°C), and thus living tissues, including embryos, do not deteriorate and can be preserved indefinitely at such temperatures. However, the formation of ice within the cell disrupts membranes and other intracellular structures, leading to a loss of cellular function and cell death after thawing. Consequently, the major task in cryopreservation is to prevent intracellular ice formation, either by removing water from the cell or by inhibiting the formation of ice crystals. Comprehensive reviews of the principles and practices of embryo cryopreservation include those of Schneider and Mazur (1984), Renard (1984), Leibo (1986, 1989, 1990), Rall (1987), Massip et al. (1987, 1989), Mazur (1990), and Niemann (1991).

Conventional equilibrium cryopreservation involves equilibration of the embryo in successively increasing concentrations of permeant cryoprotectants such as glycerol or dimethylsulphoxide, thereby reducing the amount of intracellular water. After the embryo is placed in small vials or tubes ("straws," usually used for conserving frozen semen for AI), it is slow-cooled at controlled rates (supercooling) to approximately -10°C, when ice begins to form in the extracellular medium, increasing the extracellular solute concentration, and thereby causing further dehydration of the cells of the embryo. This sequence of extracellular freezing, increasing solute concentration in the extracellular medium, and cellular dehydration continues with slow cooling to -30°C or below, the extent of dehydration increasing with decreasing cooling rate. The embryo in its container is then plunged into liquid nitrogen for storage. When required for transfer, the embryo is removed from the liquid nitrogen and thawed and warmed at a rate dictated by the cooling protocol used in the freezing procedure. The final step is to remove the cryoprotectant either by successive equilibration through decreasing concentrations of the cryoprotectant, or in one or two steps in a hypertonic solution of an impermeable solute such as sucrose.

Recently, non-equilibrium cryopreservation (vitrification) has been successfully used with cattle embryos. In this approach, embryos are exposed to high concentrations of mixtures of permeant and impermeant solutes, after which they are plunged directly into liquid nitrogen. The embryo is dehydrated to some extent, but the major effect is that the water inside and outside the cells is transformed into a non-crystalline glass-like state, rather than crystallizing. The technical simplicity, and hence rapidity, of this technique offers considerable advantages over equilibrium cryopreservation. However, the high concentrations of cryoprotectant solutions required for vitrification render the embryos susceptible to osmotic shock, and thus their exposure to the solutions must be kept brief.

Cattle, sheep, and goat embryos have all been successfully cryopreserved and can be stored indefinitely in liquid nitrogen. Horse embryos have also been successfully cryopreserved, but the success rate has been found to decrease with increasing developmental stage (Slade et al. 1985) or size (Seidel et al. 1989; Squires et al. 1989) of the embryo. The successful cryopreservation of pig embryos has been particularly difficult to achieve, and only recently have live young been produced after freezing (Hayashi et al. 1989; Kashiwazaki et al. 1991). The cryopreservation of pig semen has been equally difficult, perhaps suggesting that an unknown feature of pig cell membranes is a common factor.

Cryopreservation has become routine in commercial ET, but it is important to note that it can lead to loss of some embryos and reduced pregnancy rates after the transfer of the embryos that survive the procedure. Consequently, there remain challenges of practical importance to be resolved through basic cryobiological studies. Chief among these in cattle is the cryopreservation of oocytes, zygotes, and early cleavage stages, which will be essential to making full use of embryos produced *in vitro* (see, for

example, Parks and Ruffing 1992), and resolving the difficulty of cryopreserving embryos that have been subjected to various micromanipulations.

Though cryopreservation is essential for long-term storage of embryos, there are often circumstances in which shorter-term preservation of viability can be achieved more efficiently by simply cooling embryos (0°C to 4°C), without any cryoprotectants, to lower their metabolic rates. This approach has proved useful with cattle (Leibo and Winninger 1986; Pichavant et al. 1990) and horse (Pashen 1987; Cook et al. 1989; Carney et al. 1991) embryos. It cannot be applied to porcine embryos, which do not tolerate cooling below 15°C without protection.

Transfer of Embryos to Recipient Females

The actual transfer of embryos into suitably selected recipients, which used to be a surgical procedure, is now made almost exclusively transcervically in cattle and requires the dexterity that can only come from practice. Like collection techniques, transcervical transfer has benefited enormously from the commercial development of special apparatus in response to the demands of the ET industry. In horses, transcervical transfer is much easier than in cattle. In sheep, goats, and pigs, transfer techniques remain surgical, but laparoscopy is being quite widely adopted in the small ruminants.

As with embryo collection techniques in cattle, the commercial need for simplification in the interests of efficiency and of making repeated use of animals without risking their damage at surgery led the transition from surgical to transcervical methods of transfer. The equipment for such transfers, based on the straws and guns used for AI, has also improved enormously through the efforts of commercial suppliers; the transfer procedure is not now a serious limitation to ET in experienced hands. Nevertheless, some small ET organizations still use surgical transfers through the flank because they lead to more consistent results.

Criteria for selecting suitable recipients have been investigated extensively in commercial organizations, confirming the necessity of good synchrony between the donor, recipient, and stage of embryonic development (Hasler et al. 1987; Broadbent et al. 1991). The synchronization of estrous cycles with PGF_{2α}, progestagens, or both, has simplified recipient management, but careful observation and recording of behavioural estrus remain central to the achievement of good results. In contrast to analogous procedures in humans, the animal used as an embryo donor is never required to act as a recipient during the same, stimulated, cycle.

In mares, there has been a similar switch from surgical to transcervical transfer techniques and a similar reluctance by some practitioners to sacrifice even a marginally higher success rate to the convenience of avoiding surgery.

Transfers in the small ruminants and pigs are performed surgically, by laparoscopy in some practices. Transfer into the oviduct has advantages

in goats (Armstrong et al. 1983) and pigs (Broermann et al. 1990). For pigs, Polge and his colleagues at Cambridge have shown that two days' asynchrony does not seriously affect results provided the recipient ovulates after the donor (Polge 1985). However, only one day's asynchrony is tolerable in the other direction (Pope 1989). In pigs, it is also beneficial to group the embryos being transferred into given recipients as batches of similar developmental stages because, if the embryos differ very much in development, the more advanced tend to survive at the expense of the relatively retarded (Pope 1988, 1989). Pope (1989) has also suggested that the survival of transferred pig embryos might be improved by using synchronous pregnant recipients.

Producing Embryos by *In Vitro* Procedures

The production of embryos of domestic species by IVF offers significant advantages over superovulation and development *in vivo*, for both practical and research purposes (Bruyas et al. 1988; Gordon 1991). As in humans, IVF can be used to circumvent fertility problems caused by anatomical defects. Also, the genetic line may be preserved when valuable individual animals have to be slaughtered for any reason (Xu et al. 1992a). However, these applications are of relatively minor importance in the domestic species. Much more to the point is the possibility of producing large numbers of embryos from specific individuals or populations of animals, for several reasons. First, production of large numbers of half-sibling embryos by fertilization of ova from one cow with semen from several bulls can markedly improve and accelerate genetic testing for the ability to inherit desirable traits. Second, it may be possible to increase the proportion of morphologically normal embryos compared to those collected after superovulation, because the conditions for oocyte maturation and subsequent embryo development can be controlled *in vitro*. Third, large numbers of early-cleavage-stage embryos can be obtained for studies of early development, and for genetic and other manipulations. Fourth, large numbers of embryos of desired types can be produced at low cost from oocytes collected at slaughter. Such embryos can be used, for example, to produce beef calves, perhaps as twins, from the lesser quality cows in a dairy herd that are not required to produce replacement dairy heifers, or to quickly establish populations of European cattle breeds in developing countries by transfer into indigenous breeds.

The history of successful IVF dates to the production of live rabbits by Chang in 1959. Early work in farm species was unsuccessful (see Betteridge 1977), and it was not until four years after the birth of the first human IVF baby, Louise Brown (Steptoe and Edwards 1978), that Brackett et al. (1982) produced the first IVF calf from an oocyte that had fully matured *in vivo* (in the donor's ovary), had been surgically recovered from

the oviduct, and, once fertilized, had been transferred to the recipient oviduct as rapidly as possible to avoid the deleterious effects of holding the zygote *in vitro*. The medical and practical exploitation of IVF in domestic animals lagged behind that in human medicine for several years because physiological and practical considerations make IVF in domestic animals considerably more difficult than in humans. However, advances in oocyte retrieval, *in vitro* maturation, and *in vitro* culture have brought the technology to the level at which it is, today, widely used experimentally and commercially.

Oocyte Collection and Maturation

As in human medicine, the surgical collection of oocytes from cattle was superseded first by laparoscopic recovery of *in vivo*-matured oocytes from stimulated follicles (Lambert et al. 1986; Sirard et al. 1985), and more recently by aspiration of oocytes from all available antral follicles in stimulated or unstimulated ovaries at repeated laparoscopies or with ultrasonographically guided transvaginal puncture (Pieterse et al. 1988; Kruip et al. 1991). The success rates for ultrasonographically guided oocyte retrieval are sufficiently good that the procedure may become a practicable alternative to superovulation in specified donor animals.

In humans, oocytes are not harvested until after the pre-ovulatory LH surge; thus, oocyte maturation occurs *in vivo*. However, this requires frequent monitoring of follicular development and circulating hormone concentrations, which is highly impractical, if not impossible, in domestic animals. Oocytes can undergo spontaneous maturation when removed from the follicle, but optimization of the culture conditions for nuclear and cytoplasmic maturation of oocytes from domestic animals has required considerable effort. The technical details of studies of *in vitro* maturation are too numerous to list here, but beneficial effects of gonadotropins, steroid hormones, sera, cyclic nucleotides, and supplementary granulosa cells on the process have been demonstrated (see Sirard 1989; Gordon and Lu 1990; Greve and Madison 1991). The development of *in vitro* maturation represents a significant advance in embryo biology, considerably beyond the practices used in human medicine, and makes possible two new practical approaches to producing embryos.

First, embryos can now be produced by the *in vitro* maturation of oocytes harvested from small to mid-size antral follicles of ovaries collected at slaughter so that large numbers of embryos of uniform stages of development can be obtained at reasonable cost. Not only can this provide hitherto unobtainable numbers of embryos for research (e.g., Rieger et al. 1992), but it is also being exploited commercially to provide specific types of embryos, for the purposes described previously. In addition, it may ultimately be possible to extend these techniques to the millions of primordial germ cells, oogonia, or oocytes present in fetuses (Betteridge et al. 1989b), given that Eppig and Schroeder (1989) have

produced young by growing mouse oocytes and pre-antral follicles *in vitro* to the point where they are capable of undergoing *in vitro* maturation and IVF successfully.

Sperm Preparation and IVF

Overall, the preparation of sperm for IVF in domestic animals is similar to that used for humans and other species, involving washing of the sperm, gentle centrifugation, and "swim-up" separation of the viable sperm, followed by co-incubation with the oocytes at an appropriate concentration. However, there are two major differences in the preparation of human and domestic animal sperm. First, in most cases, frozen-thawed rather than fresh sperm is used in animals, necessitating removal of all traces of the freezing media ("extenders"), which can have deleterious effects on oocyte maturation and subsequent embryo development (Olson et al. 1992). Second, whereas the washing and swim-up procedures themselves are generally sufficient to induce sperm capacitation in humans and rodents, other measures are required to induce capacitation in domestic species. Originally, this was accomplished by short-term incubation of the sperm in hyperosmotic medium (Brackett et al. 1982), but capacitation is now usually induced by exposure to heparin during co-incubation with the oocytes (see Greve and Madison 1991).

It should be noted that, in cattle, natural breeding involves upwards of five billion sperm, contained in a single ejaculate, to produce one calf. In AI, this is reduced to the 20 million sperm in one insemination straw, a 250-fold difference. In contrast, only about 50 000 sperm are used to fertilize 10 to 50 oocytes *in vitro*. Consequently, IVF is going to be of especially great practical importance when only limited numbers of sperm are available, for example, from dead bulls or after separation of X- and Y-bearing sperm for sex selection (see below).

***In Vitro* Culture**

Whereas two- to eight-cell human embryos can be transferred into the uterus (Gerrity 1992), cattle embryos can develop in the uterus only at or after the morula stage (Newcomb and Rowson 1975). Consequently, only minimal development of human IVF embryos is required before transplantation, but, for all practical purposes, IVF embryos of domestic animals must undergo significant development before transfer to avoid the difficulty and expense of surgical transfer into the recipient oviduct. The elaboration of techniques to support the development of domestic animal embryos to the morula or blastocyst stage has, therefore, been the focus of a great deal of research, and apparently minor details have proved crucial in making significant advances. For example, raising the incubation temperature by 2°C during IVF was a key event in producing the first IVF piglets and lambs in 1983 and 1984, respectively (Cheng and Polge, unpubl.).²

For both scientific and practical reasons, culture of embryos in totally defined media and conditions would be most desirable. However, in all species, except some strains of mice, the development of early-cleavage-stage embryos under conventional culture conditions is blocked at a stage approximately coincident with the activation of the embryonic genome or MZT (Telford et al. 1990). The fundamental causes of these blocks are unknown but are associated with deficiencies in transcription of the embryonic genome and subsequent protein synthesis (Eyestone and First 1991), possibly resulting from the toxic effects of intracellular oxygen radicals (Rieger 1992). Mouse and hamster embryos can be successfully cultured through their potential blocks in certain defined media (see Leese 1991). However, only limited success has been achieved for the culture of pig (Reed et al. 1992), cow (Bavister et al. 1992), and sheep (Walker et al. 1992) embryos through their respective developmental blocks in defined media. In the study by Walker et al. (ibid.), *in vitro* culture of sheep embryos was associated with significant increases in length of gestation, birthweight, fetal deformities, and perinatal mortality.

These studies underline the fact that the defined media and physical conditions currently used for the culture of embryos of domestic species are inadequate to completely support development. Current efforts to improve culture include studies of the relationships between development of domestic animal embryos and energy substrates (Gardner and Batt 1991; Thompson et al. 1992; Rieger et al. 1992), oxygen (Thompson et al. 1990; Batt et al. 1991), and phosphate (Pinyopummintr and Bavister 1991). Of particular significance are observations that early embryos are capable of producing, have receptors for, and can respond to, a number of growth factors, suggesting that autocrine or paracrine mechanisms are involved in early development (Schultz and Heyner 1993). A notable example is the very recent report that platelet-derived growth factor promotes development of bovine embryos through the eight-cell block in culture, and transforming growth factor alpha or fibroblast growth factor promote subsequent blastulation (Larson et al. 1992).

As an alternative to *in vitro* culture, an initial approach was to transfer groups of early-cleavage-stage embryos temporarily into the oviducts of sheep or rabbits. The embryos were then collected from these intermediate recipients at the morula or blastocyst stage, several days later, for trans-cervical transfer of individual embryos into the uteri of foster mothers. Although less technically demanding than direct surgical transfer of each embryo into the oviduct of a recipient cow, this technique is still inconvenient and the recovery rates from the intermediate recipients are highly variable (Greve and Madison 1991).

Co-culture with somatic cells is a compromise between totally defined culture and the use of intermediate recipients, and has proved to be the most successful approach to culturing embryos through their potential developmental blocks. Co-culture with a variety of cell types, including granulosa cells, trophoblast cells, fibroblasts, epididymal epithelial cells,

uterine cells, and, in particular, oviductal epithelial cells, has been shown to be beneficial to the development of embryos of several species (Rexroad 1989; Xu et al. 1992b). The mechanism by which these diverse somatic cells exert their beneficial effects is not known. Although it has been suggested that the somatic cells secrete one or more tissue-specific cellular factors (Gandolfi et al. 1989), more widely distributed cellular growth factors may be involved (Pollard 1992). However, it is equally likely that the somatic cells simply serve to remove inhibitory compounds from the medium (Bavister et al. 1992). Whatever the mode of action, co-culture has proved to be an effective method of producing large numbers of embryos, particularly cow embryos, and is likely to be used for the foreseeable future. In one study from our own laboratory, for example, 65 percent of two-cell embryos developed to the hatching or hatched blastocyst stage in co-culture with bovine oviductal epithelial cells (Xu et al. 1992b). The *in vitro* development of human embryos has been shown to be significantly improved by co-culture with Vero epithelial cells, derived from Green monkey kidneys (Ménézo et al. 1990). These authors suggested that routine co-culture to the blastocyst stage could lead to improvements in the pregnancy rates in human IVF programs, because developmentally incompetent embryos could be identified and eliminated, and those that survived the culture would be at a uterine stage of development at the time of transfer. An even simpler approach to exploiting the potential benefits of co-culture may be to use the embryos themselves as growth-promoting cells because the development of embryos is markedly improved when the embryo/culture volume ratio is increased (Rieger and Betteridge 1989).

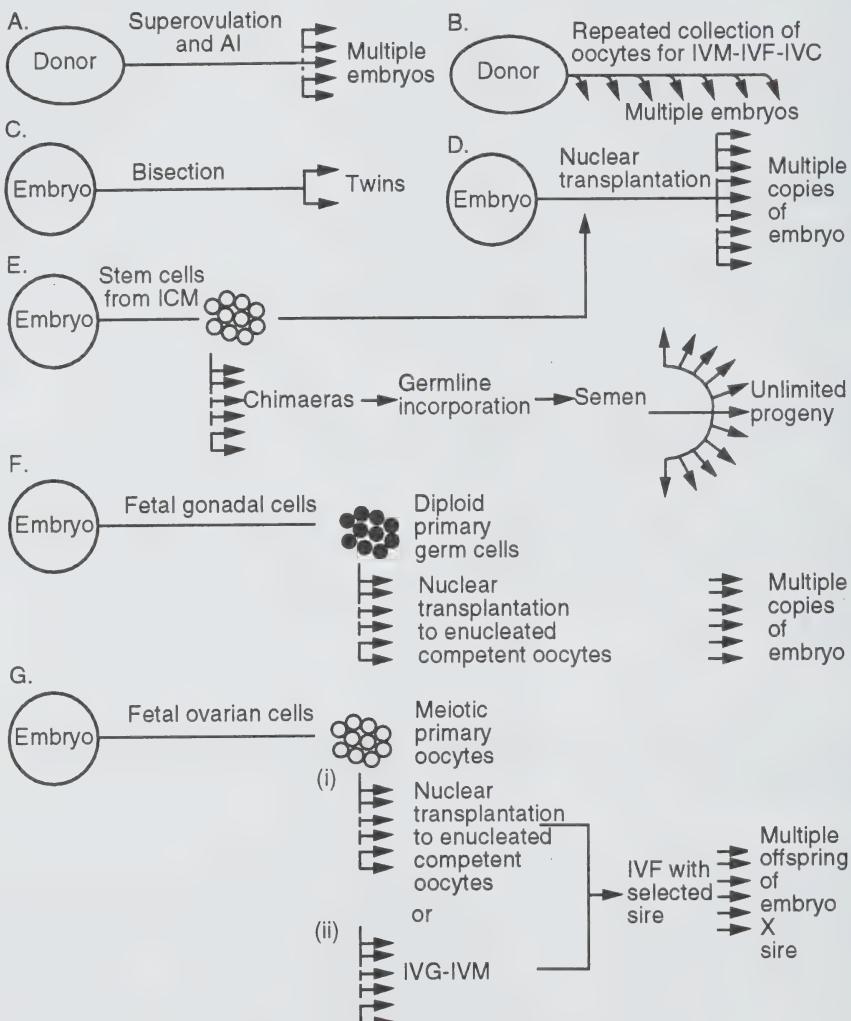
Specialized and Experimental Techniques of Embryo Manipulation

Micromanipulation of Embryos

Micromanipulation refers to techniques in which early embryos or gametes are structurally or functionally altered using minute, specialized instruments while being viewed through a microscope. Recent progress in the micromanipulation of embryos has been remarkable (Fehilly and Willadsen 1986; Picard and Betteridge 1989; Loskutoff 1990) and is a key component of current procedures in embryo sexing, "cloning," and gene transfer (see Figure 4 and below).

Broadly speaking, embryos can be either divided or combined at various developmental stages up to and including the blastocyst stage. The simplest application is bisection, in the form of "embryo splitting," to produce limited numbers of genetically identical individuals. This has been

Figure 4. Alternative Approaches to Producing More and Cheaper Embryos or Progeny of Desired Genotypes



Note: A to D are already in practice; E to G are hypothetical; D to G are potentially most useful in combination with methods of producing transgenic livestock.

Source: Betteridge 1990a.

used commercially to a limited extent for several years. More recently, this technique has been extended to produce viable embryos and calves from separated blastomeres from 4- to 16-cell cattle embryos (Loskutoff et al. 1993). Combinations of cells from different embryos can be used to form chimaeras in which, for example, the inner cell mass and the trophoblast come from different individual embryos of the same or different species. This procedure is of potential significance to the preservation of endangered species because it allows such embryos to be nurtured in the uterus of different, but closely related, species. Alternatively, the chimaerism may extend to the inner cell mass and thence to the fetus and resultant animal. This form of chimaerism is important as a research technique and could become relevant to production techniques as well (Picard et al. 1990). Experimentally, it is through chimaeras that embryonic stem cells have been introduced into the germline in mice after making them transgenic *in vitro* (Robertson 1991). This seems to be one of the most promising routes to controlled transgenesis in the farm species as well (see below). For future production, quite apart from future applications involving transgenesis, stem cells (which have not yet been produced in cattle) could be one way of proliferating the germline of a particular embryo. The approach involving chimaeras for this purpose (Figure 4E) may eventually be superseded by producing entire animals from the stem cells, as is now possible in mice (Nagy et al. 1990). In addition, perhaps trophoblastic tissue identified as being especially well endowed with factors promoting the maintenance of pregnancy could be combined with inner cell masses of particularly valuable embryos to improve their survival.

These micromanipulative techniques for production purposes have three major limitations. The first is the cost of the skilled labour and specialized facilities required. However, the transition from the laboratory to the field has been made quite readily and economically for the simpler methods, such as embryo splitting, and even for some of the more complex, such as sexing (see below). The second limitation is the reduced survival of manipulated embryos after cryopreservation, but many efforts are being made to correct this shortcoming. The third is the inevitable puncturing of the zona pellucida, which, as discussed in a previous section, currently renders the embryo ineligible for export. This is ironic because one application of removing a few cells by biopsy could be to monitor individual embryos for infectious or metabolic diseases.

Sex Selection

The ability to preselect the sex of offspring would be of substantial benefit to livestock producers in a variety of situations. For example, the ongoing success of commercial dairying requires a continual supply of heifers to replace old or less productive cows, while bull calves are seldom of any economic value (Betteridge 1989b). In traditional cow-calf beef operations, bull calves are considered more desirable because of their

higher rates of gain, while heifer calves are required in specialized beef production schemes (Taylor et al. 1985, 1986).

Without question, the separation of X- and Y-bearing spermatozoa, if possible, would be the best approach to sex selection. Numerous techniques have been purported to separate X- and Y-bearing sperm, but, to our knowledge, only flow cytometry has withstood rigorous scientific analysis. In rabbits, flow cytometry has been shown to separate sufficient spermatozoa to be used successfully for AI *in vivo* (Johnson et al. 1989). Even if this does not prove to be a practical means of providing the numbers of spermatozoa that would be required for AI in the large domestic species, it may be sufficient to be used for IVF or, in very special cases, for IVF by sperm injection, a procedure recently shown to be capable of producing calves from immotile spermatozoa (Goto et al. 1990). Field trials using bovine embryos derived from IVF with X- and Y-bearing sperm separated by flow cytometry are in progress (Johnson 1992).

The second approach to sex selection in domestic animals is by determination of the sex of early embryos before transfer. The need for embryo sexing and the price that could be paid for a reliable procedure have been defined (Betteridge 1989b; Ferris and Troyer 1987), and at least four methods are in use or under investigation (van Vliet et al. 1989).

The first is cytogenetic sexing, in which a biopsy or hemisection of the embryo is cultured in the presence of Colcemid to arrest the cells in metaphase. The cells are then spread on a slide, fixed, stained, and examined for the presence of two X chromosomes or one Y chromosome. This technique was first used for elongated cattle embryos (Hare et al. 1976), and later elaborated to allow the analysis of earlier stages (King et al. 1979; Picard et al. 1985). The results of cytogenetic sexing are generally unequivocal, but the technique is limited by the mitotic activity of the embryo so that, even in the most skilled hands, the success rates rarely exceed 80 percent (e.g., Xu et al. 1992c).

The second method relies on the expression of sex-specific antigens on the embryonic cell membrane. Although evidence suggests that there are both male- and female-specific antigens, the focus in embryo sexing has been on the male-specific H-Y antigen. Male-specific antisera have been used in cytotoxicity or immunofluorescence assays to destroy or identify the male embryos. Both assays are non-invasive and technically quite simple. However, the cytotoxicity assay is useful only for obtaining female embryos, and their viability is also compromised by the treatment, while the accuracy of the immunofluorescence assay is limited due to variable non-specific binding and the subjective nature of the interpretation of fluorescence. Consequently, like cytogenetic sexing, immunological sexing has not, so far, passed into commercial practice (Anderson 1987; White et al. 1987; van Vliet et al. 1989; Booman et al. 1989; Avery and Schmidt 1989). However, a promising alternative use of anti-H-Y antibodies for sexing embryos is that of Utsumi et al. (1991). This group has used absorbed

antisera to detect differences in the responses of male and female rat morulae to a brief (six-hour) exposure to the antibodies *in vitro*; development of males is reversibly retarded, whereas females continue to develop into blastocysts. Sex prediction in this fashion has been proved to be largely correct by ET in rats. The same Japanese group has published preliminary results of analogous work in cattle and other domestic species (e.g., Utsumi et al. 1984), and the efficacy of the method has apparently also been proved with transferred bovine embryos. Although the lack of species specificity for this approach might be seen to have special relevance to human work, the differential effect has, so far, been demonstrated only at the morula-blastocyst transition phase, later than most human embryos are returned to the patient.

Third is the application of techniques of molecular biology to identify the presence or absence of male-specific (i.e., Y chromosome) DNA sequences. A few cells are removed from the embryo and the DNA is extracted, amplified by the polymerase chain reaction, and tested with Y chromosome-specific DNA probes (Bondioli et al. 1989; Handyside et al. 1989; Herr et al. 1990; Kohen et al. 1990; several papers in *Reproduction in Domestic Animals* 1991). Results are virtually unequivocal, and the technique has even been used "on-farm" (Glasgow 1989). A major advantage to this approach is that other DNA probes can be used at the same time in order to screen the embryos for genetic diseases such as malignant hypothermia in pigs (MacLennan et al. 1990), or to select for economically important production traits (see next section, "Marker-Assisted Selection"). However, it is still technically demanding, often requires species-specific probes, and suffers from making embryos ineligible for export under existing regulations. For these reasons, considerable incentive remains to develop non-invasive methods of sexing.

Fourth, it may be possible to distinguish between male and female embryos by comparing quantitative differences in metabolic activity in perhaps two ways. For several days in early embryonic life, female embryos have two active X chromosomes; consequently, the activity of X-linked enzymes is greater in female embryos than in male embryos (Rieger 1984). This method has been shown to have promise in mice (Williams 1986), but is not yet practicable in cattle (Tiffin et al. 1991). However, male embryos develop faster than female embryos in mice (Tsunoda et al. 1985) and cattle (Avery et al. 1991; Xu et al. 1992c); this is reflected in the rate of glucose metabolism (Tiffin et al. 1991). It may be possible to differentiate between male and female embryos by measuring the rate of uptake of glucose or some other metabolic substrate.

This very early differential development rate according to sex is of extraordinary significance and an excellent example of serendipity made possible by the *in vitro* techniques described herein. The facts argue strongly against the generally accepted Jost hypothesis of phenotypic sexual differentiation being entirely secondary to testicular differentiation,

as is discussed in more detail elsewhere (Mittwoch 1989; Xu et al. 1992c). Presumably, then, X- and Y-specific genes are actively controlling factors involved in the very earliest cleavages of mammalian embryos. Identification of these factors could conceivably pave the way to controlling phenotypic sex independently of genetic sex, which could be a powerful tool in animal production.

It is also possible to select the sex of offspring by fetal sex diagnosis and selective abortion (Leibo and Rall 1990). As in humans, the sex of the fetus can be determined in early pregnancy by karyotypic or Y-probe analysis of amniotic cells, or by determination of the location of the genital tubercle by diagnostic ultrasound (Curran 1992). The induction of abortion at such stages in domestic animals is routine, and there is little risk of medical complications.

Marker-Assisted Selection

In traditional animal breeding, the selection of breeding animals relies on evaluating the phenotype of individuals or their offspring for economically important traits such as milk or meat production and disease resistance. For example, the genetic merit of a dairy bull is determined by measuring the amount and quality of milk that his daughters produce. Recent advances in molecular genetics, however, offer the possibility of selecting breeding animals that carry the favourable alleles for genetic loci determining economically important traits. This approach is known as "marker-assisted selection" (Georges 1991; Georges and Massey 1991; Wilmut et al. 1992b), and is based on the identification of a large number of polymorphic DNA marker sequences. Although technically similar to the approaches used to identify male embryos or carriers of genetic diseases by specific DNA probes, marker-assisted selection is considerably more complex because economically important traits are often the result of many individual genes. Consequently, large numbers of animals have to be genotyped in order to determine which DNA polymorphisms are useful markers for any given trait. However, once suitable markers have been identified, marker-assisted selection can be used along with conventional methods to increase the rate of genetic gain. Another advantage is that the genetically most desirable animals could be identified at a very young age.

Georges and Massey (1991) have suggested that this approach could even extend to analysis of fetal oocytes, in a breeding strategy they have termed "velogenesis." In this scheme, a cow carrying a desirable trait would be mated with a bull from a (recipient) line into which the desired trait is to be introduced. Oocytes would be obtained from the resultant female fetuses as early as 90 to 180 days of gestation, and matured and fertilized *in vitro* with sperm from a bull from the recipient line, to produce back-cross embryos (Betteridge et al. 1989b). The embryos carrying the markers would then be transplanted to recipient cows, and fetal oocytes again collected at 90 to 180 days of gestation to repeat the cycle. Several

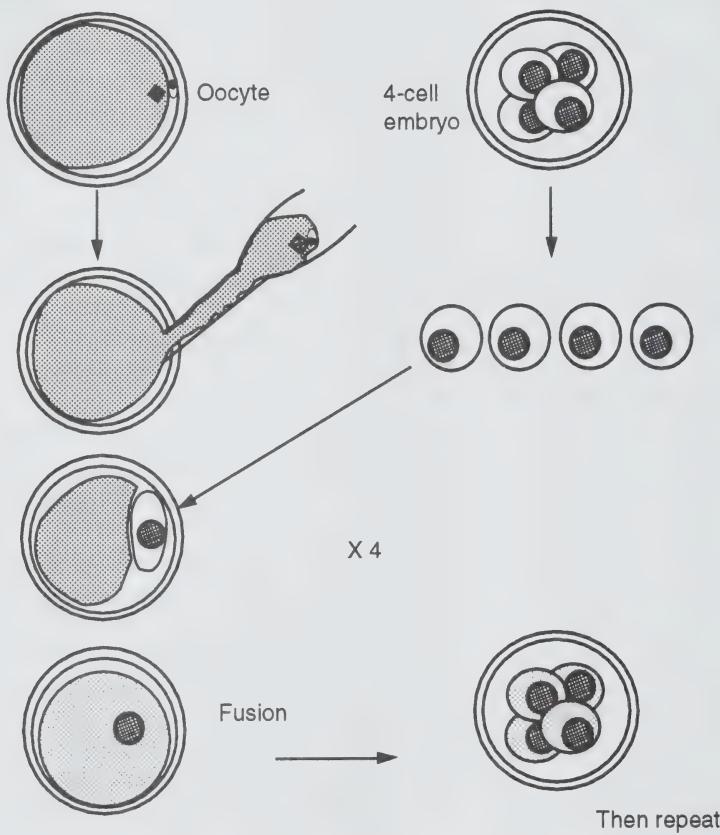
such cycles, each representing a generation interval of less than six months, would result in a very rapid introduction of the desirable genes into the recipient line (Georges 1991).

Cloning

Forty years ago, Briggs and King (1952) showed that tadpoles could develop from embryos produced by the transfer of nuclei from frog blastula cells into enucleated oocytes. Since then, developmentally competent embryos have been produced by similar cloning techniques in other amphibians, fish, mice, rabbits, sheep, pigs, and cattle (Picard and Betteridge 1989; Robl and Stice 1989; Willadsen 1989; Prather and First 1990; Smith and Wilmut 1990; Bondioli et al. 1990; Renard 1990; Westhusin et al. 1991). Embryo cloning in domestic animals is achieved by using variations of the technique described by Willadsen (1986), which are reviewed in Prather and First (1990) and Barnes et al. (1991). Briefly, recipient oocytes are pretreated with cytochalasin and then enucleated or bisected to remove the chromatin. Single blastomeres are taken from 4- to 32-cell embryos, and placed inside the zona pellucida of the recipient oocyte, tightly against the oocyte cell membrane. Sendai virus or, more usually, an electrical pulse is used to induce the formation of pores between the blastomere and oocyte, ultimately resulting in fusion of the two structures (Figure 5). The resultant embryo clone is thus composed of the nuclear genetic material of the mother and father of the original embryo, and the cytoplasmic structures and contents from the recipient oocyte, usually from a different female. The functional significance of the procedure is, of course, that each of the blastomeres from the original embryo can be used to produce a clone, yielding, in the first generation, as many as 30 or more embryos with exactly the same nuclear genotype. Moreover, after development through several cell divisions, the process can be repeated over successive generations. Consequently, there is theoretically no limit to the number of copies of an embryo that might be produced in this way. However, it is important to note that somatic cells of adult animals with proven characteristics cannot yet be used to produce "clones" in this way.

Cloning by this technique has been in commercial use in at least three large companies. However, recent reports from those commercial operations (of which the most prominent has gone bankrupt) have revealed a disturbing tendency toward increased abortion rates, excessive birthweights, birth defects, and perinatal mortality in calves arising from cloned embryos (Bondioli 1992; Stice 1992; Willadsen et al. 1991). It is unclear whether these problems arise from some specific aspect of cloning, or from the subsequent *in vitro* culture, as has been observed in *in vitro* fertilized sheep embryos (Walker et al. 1992), but they will have to be rectified before cloning can become routine.

Figure 5. The Principles of Nuclear Transplantation for the "Cloning" of Embryos



Note: Oocytes are enucleated by aspiration with a micropipette. Each separated blastomere (four for illustrative purposes) is fused (usually by electrofusion) with the cytoplasm of an enucleated oocyte. Each identical nucleus therefore programs the development of an identical embryo, which can be used either to repeat the process or for transfer to a recipient.

Source: Betteridge 1990b.

A further concern relevant to the commercial application of cloning in domestic animals is the contribution of the recipient oocyte to the ultimate production characteristics of the clone. Traditionally, production traits have been thought to be associated solely with the nuclear genes, and no attention has been paid to the animals from which the recipient oocytes are obtained. However, it has recently been shown that, in dairy cattle, significant differences in traits of milk production and reproduction are associated with differences in mitochondrial DNA (Freeman and Beitz 1992). Consequently, it may be necessary to evaluate both the nuclear genetics of the original embryo and the mitochondrial genetics of the recipient oocyte to obtain the maximum gain in milk production or other traits.

Pluripotent embryonic stem cells (derived from the inner cell mass of blastocysts) and primordial germ cells have also been proposed as sources of nuclei (or pronuclei) for cloning in domestic animals. However, due to parental imprinting or successive gene deactivation, pluripotency decreases with development until it is lost at the primitive ectoderm stage. Similarly, recombination of two male or two pronuclei within an oocyte to produce androgenotes or gynogenotes, respectively, is incompatible with development because of imprinting (Wilmut et al. 1992a).

Gene Transfer

Ten years ago, Palmiter et al. (1982) showed that injection of the rat growth hormone gene into one-cell mouse embryos led to supranormal growth after birth. This report was immensely significant to all areas of the life sciences, but particularly to animal scientists because increased growth rates are generally considered a highly desirable production trait. Major efforts were therefore directed toward achieving a similar result in domestic animals, and these studies led to the successful transfer, incorporation, and expression of human and bovine growth hormone genes in pigs (Pursel et al. 1990) and sheep (Nancarrow et al. 1991; Rexroad et al. 1990). As hoped, the transgenic pigs showed increased growth, rates of gain, and feed efficiency (Pursel et al. 1990). In addition, in both the pigs (*ibid.*) and sheep (Rexroad et al. 1990), the subcutaneous fat was reduced and muscle increased, another highly desirable characteristic. However, the persistent circulating growth hormone also caused severe physiological defects, including lameness, lethargy, gastric ulcers, and infertility in the pigs (Pursel et al. 1990) and diabetes leading to death in the sheep (Rexroad et al. 1990).

The fundamental cause of these problems appears to have been associated with the metallothionein promoter used in conjunction with the transgenes. Growth hormone production is developmentally regulated and normally tightly controlled by feedback mechanisms. In contrast, metallothionein production is turned on at all stages of development. Consequently, growth hormone was produced at inappropriate times,

leading to the health defects. Having recognized this problem, more appropriate and controllable promoters are now under investigation.

The other major area of interest in transgenic domestic animals is the possibility of producing novel proteins in milk, particularly pharmaceutical compounds of significance to human medicine. In the popular press, this has become known as "genepharming." Although many therapeutically important proteins such as human insulin and growth hormone can be produced by transgenic prokaryotes, many complex proteins cannot, because the prokaryotic hosts lack the appropriate mechanisms for post-translational processing. To promote the production of a desired protein in milk, a mammary-gland-specific promoter gene, such as β -lactoglobulin, is used to ensure that expression of the transgene is in the proper place at the proper time. The genes for human blood coagulation factor IX and α_1 -antitrypsin have been transferred into sheep, and measurable quantities of the proteins have been found in the milk (Wilmut et al. 1990).

Wilmut et al. (1991) provided an extensive review of the procedures of genetic manipulation and its implications for research in reproductive biology.

Measures to Reduce Early Embryonic Mortality

Early embryonic death in inseminated cattle is well known to be a major cause of infertility and has been the subject of numerous books and reviews (e.g., Sreenan and Diskin 1986; Shemesh and Weir 1989; Roberts et al. 1990; Albihn 1991). It is often considered to be inevitable — "a normal way of eliminating unfit genotypes in each generation" (Bishop 1964). Nature, however, has not found it necessary to use such a strategy in some wild ungulates; pregnancy rates late in gestation in over 4 000 mature reindeer, for example, exceeded 99 percent (Godkin 1986). Thus, there are circumstances, in the wild, in which breeding at a single estrus will almost ensure the production of a calf. How domestication has changed those circumstances so detrimentally can only be guessed at, but experimental evidence in mice indicates that free-choice mating favours the maintenance of major histocompatibility complex (MHC)-genotype heterozygosity (Potts et al. 1991; Howard 1991), which could, perhaps, be of selective advantage for embryonic and fetal survival.

Losses of transferred embryos can be looked upon as a special category of early embryonic death and are more pronounced soon after transfer than at later stages in both cattle (Markette et al. 1985) and horses (Villahoz et al. 1985); there is great concern over this phenomenon and how it might be reduced or eliminated (Betteridge and Loskutoff 1993). In contrast to the situation in humans, cytogenetic causes are likely to account for a relatively low proportion of early pregnancy failures; the incidence of chromosomal abnormalities in domestic animal embryos generally falls in the 10 to 15 percent range (King 1990). Considerable

progress has been made in understanding how the embryo and its maternal environment interact during early pregnancy (Bazer et al. 1991; Harney and Bazer 1989; Nephew et al. 1991; Roberts 1991; Zavy et al. 1982), and this could lead eventually to soundly based therapy. Meanwhile, treatments aimed at reducing embryonic loss remain largely empirical and unproven. One approach to augmenting pregnancy rates after transfer has been to supplement recipients' endogenous progesterone levels either directly, with progesterone administered by injection or released from intravaginal devices, or indirectly, by stimulating luteal activity with hCG or Gn-RH. Neither progesterone nor hCG has given consistently encouraging results in recipients (Sreenan and Diskin 1987). In more recent studies, progesterone released from an intravaginal device beginning six to eight days after insemination increased pregnancy rates significantly in some studies (Macmillan et al. 1991) but not in others (Van Cleef et al. 1991). Nevertheless, in New Zealand it is now not uncommon to use intravaginal progesterone in recipient sheep. Similarly, a Gn-RHa administered 12 to 14 days after AI has been reported to augment pregnancy rates in some studies, particularly in cows previously synchronized with PGF_{2 α} (Lajili et al. 1991; Ryan et al. 1991), but to be completely ineffective in others (Jubb et al. 1990; Thatcher et al. 1993). It remains to be seen if either treatment will be beneficial in recipient cattle.

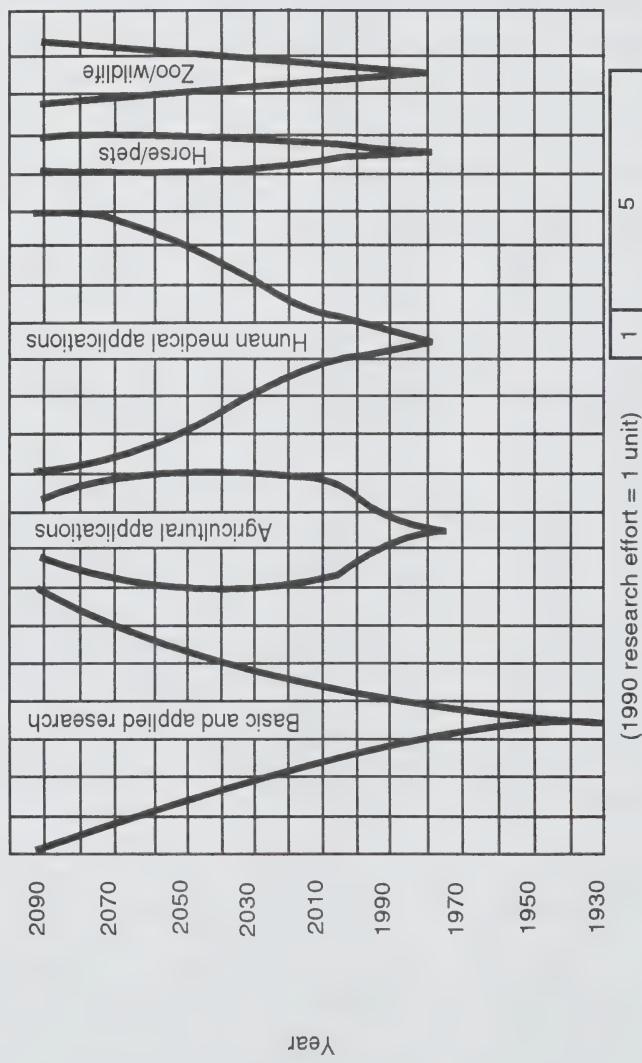
Higher pregnancy rates are also being obtained in normally bred sheep after systemic injections of interferons, which may mimic some of the effects of conceptus secretions (Nephew et al. 1990). Conceptus secretions themselves may also be useful in improving pregnancy rates obtained with transferred embryos; co-transfer of trophoblastic vesicles (made by cutting up trophoblastic tissue from elongating hatched embryos that produce the secretions) with frozen-thawed embryos has been reported to improve pregnancy rates by about 10 percent (Heyman and Ménézo 1987).

The patterns of embryonic loss after transfer (particularly of manipulated embryos; see "Cloning" section above) may possibly differ somewhat from patterns of loss after normal insemination (Betteridge and Loskutoff 1993). If so, it could be important, because the deficiencies involved would be different in embryos and fetuses lost at different stages. Any effort to understand, remedy, and avoid such deficiencies will therefore need to take this into account.

Future Directions

As shown in Figure 6, Seidel (1991) has predicted that basic and applied research on preimplantation embryos will increase sixfold over the next 100 years, and be closely followed by increasing application of the technology to agriculture, human medicine, pets, and wildlife species. The

Figure 6. Projected Worldwide Growth of Scientific and Technologic Work with Preimplantation Embryos



Source: Seidel (1991), with permission of the author and *Theriogenology*.

applications in domestic animals can be divided into two general directions: (1) accelerated selection and improved use for human benefit of animals on the basis of their natural characteristics, and (2) genetic modification to produce animals with particular new characteristics.

As noted previously, ET has already made a significant contribution to the rate of genetic gain for naturally occurring production characteristics, particularly in dairy cattle (see C. Smith 1986, 1988, 1989). However, the rate of genetic gain will increase even more rapidly by the implementation of more sophisticated breeding strategies that rely on ET and related techniques that are already available (see Christensen 1991). Examples are the establishment and intensive selection of nucleus herds of elite animals through multiple-ovulation ET programs (see Ruane and Smith 1989; McGuirk 1989), and the production of multiple identical offspring by embryo cloning, and full- or half-siblings by IVF. For the purposes of production, offspring of selected sex and uniformity among identical animals would simplify management, increase productivity, and improve product consistency. Given the increasing liberalization of international trade, and the fact that such approaches are already being introduced into commercial application in other countries, the Canadian livestock industry will almost certainly have to adopt these new breeding strategies and techniques to remain competitive.

The possibilities for future basic research are virtually limitless, but, as suggested by Seidel (1991), include the production of animals identical to a single parent animal by cloning of somatic cells, gynogenesis, or androgenesis, and development to term *in vitro*.

The use of gene transfer to produce animals with new characteristics is still largely under investigation, but has major implications for food production and human medicine. For example, genetic manipulation of dairy cattle to produce lactose-free milk would make this important protein source palatable to large numbers of lactose-intolerant humans, particularly in developing countries. Modifications to produce milk and meat with less fat (or different fats) and more protein may offer some health advantages for the consumer. The transfer of genes associated with resistance to specific disease could significantly reduce both production losses and the need for antibiotics in animal production. Transfer of human genes to domestic animals to produce pharmaceutically important proteins in milk could offer an almost limitless supply of these compounds for treatment of diseases such as haemophilia.

The application of ET and related techniques may offer the best and, in some cases, the only approach to the preservation of endangered species (Wildt et al. 1992). An extreme speculative example is the possibility that extinct species such as the wooly mammoth could be re-established using genetic material obtained from frozen specimens (Seidel 1991).

In practical terms, the market for ET services in the future can be expected to become more selective and competitive as cryopreserved clones of embryos of known sex and phenotype become available, first in cattle but

then in other species too. This availability is likely to depend on rather specialized laboratory facilities and personnel, so we can anticipate a considerable degree of centralization of embryo suppliers and a much wider network of ET technicians serving the market.

“Technology Transfer” Between the Medical and Veterinary Users of Applied Embryology

The short history of medical work with embryos since the late 1970s, and the slightly longer history of their veterinary use since the 1950s, share common roots in fundamental scientific investigations of reproductive physiology and embryology in laboratory species, domestic animals, and humans. Many components of successful human IVF procedures today are derived from animal studies: the basic principle of ovarian stimulation; the handling, culture, and cryopreservation of eggs, sperm, and embryos; the biopsy of embryos for genetic diagnosis; gamete intrafallopian transfer and zygote intrafallopian transfer procedures, for example. The observation (made in monkeys) that early-cleavage-stage embryos of primates, unlike those of other mammals, can survive transfer to the uterus is fundamental to human IVF. The first successes with frozen-thawed human embryos were owed entirely to procedures developed in domestic animals. However, the transfer of technology has not been only from animal work to human applications; the first successes in human IVF spurred renewed efforts to develop the techniques in domestic animals, and the use of endoscopy and ultrasonography during IVF procedures in humans has directly stimulated their analogous application in animals. In other words, there has already been a great deal of interdependence between the medical and veterinary aspects of embryo technology.

The potential for future technology transfer is at least as great as that which has already occurred. Throughout this report, we have mentioned areas of interest common to human and veterinary medicine that will almost certainly lead to, and benefit from, such exchange:

- the use of recombinant somatotropin and Gn-RH antagonists, and regulation of the dominant follicle to improve follicular development in response to exogenous gonadotropins;
- the evaluation of embryo viability with regard to transfer, based on metabolic activity;
- disease control through ET;
- the improvement of techniques of cryopreservation, particularly of oocytes;
- *in vitro* oocyte maturation, especially oocytes from pre-antral follicles;

- *in vitro* culture of embryos in defined media or with somatic cells to improve viability; and
- genetic diagnosis by molecular techniques.

Interestingly, the movement of personnel is one of the major ways in which information and technology are transferred between animal and human work. Many pioneers and leaders in human IVF developed their expertise with animals: R.G. Edwards has already been mentioned; A.O. Trounson, Jacques Testart, and Yves Ménézo are other prime examples. In addition, the demand for "embryologists" to work in human IVF clinics (evident from advertisements in scientific and clinical journals) is largely met by scientists (including two from our laboratory) who have earned a graduate degree by working with animal embryos. The importance and mutual benefit of scientific exchange between workers in the human and animal embryo fields are probably best illustrated by the extent of cross-reference between the fields in the scientific literature.

The extent to which any technique is transferred from animal to human application, particularly those involving genetic manipulation, must be evaluated in light of what is ethically and socially desirable; obviously the goals to be met are different in humans. These kinds of considerations are at the core of this Commission's mandate. In separating the possible from the desirable, it must be borne in mind that each and every technique that can be applied to domestic animal embryos before they hatch from the zona pellucida could also be applied to the human embryo. The only major differences between the possible in animals and in humans are those governed by physiological differences (see the section "Fertilization and Early Embryonic Development").

It must also be appreciated that practical applications have only recently become important motivators of embryological research, and it is important to realize that the value of such research extends far beyond generating new methods of producing, manipulating, and transferring embryos in humans or animals. Consequently, it would be wrong to suggest that future research in mammalian embryology should be planned around if and how a given project relates to a technique that should or should not be applied to animal breeding or clinical medicine. Comparative studies of the new reproductive technologies, which depend on technology transfer between the human and animal sectors, must be seen in their context — that is, as a component of broader studies of reproductive biology.

The importance of such studies has been recently emphasized by an unprecedented joint statement by the U.S. National Academy of Sciences and the Royal Society of London on global problems (Maddox 1992). The statement gives central attention to how the estimated growth of the world's population by 100 million a year is linked with human activities producing "major changes in the global environment." As means of mitigating these problems, the academies back (in order) new generations of contraceptives;

alternative energy sources; improved agriculture, animal, and plant genetics; biotechnology; and public health (including vaccines against infection such as malaria and acquired immunodeficiency syndrome). Four of these five approaches will depend on, or benefit from, embryological research, and so the paradoxical relationships between efforts to aid and hinder reproduction in animals and humans need to be clearly appreciated. They are vital to our future.

Conclusions

We are conscious of the fact that any attempt to review this wide and ever-expanding field will, inevitably, suffer from superficiality in some parts, too much detail in others, and considerable imbalance throughout, reflecting the biases of the reviewers. Another, also inevitable, danger stems from the format of the presentation; categorizing the information under headings chosen for convenience could well make it difficult to see the wood for the trees. The trees are important and impressive enough, but it is the wood — the overall advances in our knowledge of comparative reproductive biology that are resulting from the new reproductive technologies — that will be the more important to society in the long run. What could be of more fundamental importance to biology and medicine than a fuller understanding of how two haploid cells can unite to form a single diploid cell capable of differentiating into Northern Dancer, Albert Einstein, or Mother Teresa? How can the aberrant development of a neoplastic cell be understood without investigation of the mechanisms controlling differentiation so exquisitely in normal cells during embryonic development?

Another generalization that risks being lost through categorization is the need to pay due attention to animal welfare in deciding what use to make of these techniques. There can be no justification for inflicting unnecessary surgery or the difficulties of birthing oversized calves on recipients. On the other hand, should all work on transgenesis cease because early progeny were indisputably unhealthy?

We hope that this document has preserved the general essence of the subject and will provide the interested reader with enough references to enter the literature for any particular area in which more information is needed. Factual information, of course, needs to be kept central during discussions of the new reproductive technologies and their use.

To use this material in making moral or ethical judgments, or in taking philosophical or legalistic positions, is well beyond our remit. However, as concerned scientists and members of society, we recognize public apprehensions (see Betteridge 1986, 1990a, 1990b) but are convinced that research into mammalian reproduction is an essential component of humankind's need to confront the twin problems of

increasing the efficiency of animal production and decreasing the rates of human reproduction.

The questioning of whether, and how, reproductive techniques should be used is by no means new. Sir John Hammond was upbraided by the Church, on the one hand, and by cattle breeders, on the other, for advocating the use of AI (Hammond 1962). The bishops of the Church of England saw the technique as being unnatural and immoral; the cattle breeders saw it as undermining their income from breeding bulls. Some 50 years later, in 1992, a Japan Prize was awarded to Dr. C. Polge for making it possible to freeze semen, thereby transforming AI into the single biggest factor in effecting genetic improvement in cattle and feeding countless millions. The bishops' concern that AI in cattle would undermine human morality seems inconsequential in retrospect (evil eugenic practices need no new reproductive methods, as was so disastrously seen in Nazi Germany), and the sons and daughters of the cattle breeders are happily benefiting from what seemed to their fathers a threat.

Also in 1992, Dr. Polge directed a commercial company that is selling bovine embryos produced by *in vitro* maturation, fertilization, and culture to farmers in several countries and will offer them sexed embryos very soon. Whether the future impact of this type of new reproductive technology will be as great as that of AI only time will tell, but history would certainly suggest that we need to provide the opportunity to let it happen.

Acknowledgments

We thank Ms Adrienne Randall for her help with the literature search; Dr. Stanley P. Leibo, Dr. Charles Smith, and Dr. Alan G. Wildeman for advice on portions of the report in their areas of expertise; Dr. Marie-Cecile Lavoie for her critique of the entire report; and Mrs. Pat Fowle for preparation of the manuscript.

Notes

1. Drs. de la Fuente and King are at the Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph.
2. Professor Polge is at Animal Biotechnology Cambridge, Ltd., Cambridge, U.K.

References

Ainsworth, C.G.V., and J.H. Hyland. 1991. "Continuous Infusions of GnRH Advance the Seasonal Onset of Oestrous Cycles in Thoroughbred Mares on Australian Studfarms." *Journal of Reproduction and Fertility* (Suppl. 44): 235-40.

Albihn, A. 1991. "Maternal Influence on the Early Embryonic Development in the Bovine: With Special Emphasis on Repeat Breeder Heifers." Ph.D. dissertation, Swedish University of Agricultural Sciences, Uppsala.

Anderson, G.B. 1987. "Identification of Embryonic Sex by Detection of H-Y Antigen." *Theriogenology* 27: 81-87.

Armstrong, D.T. 1991. "Factors Influencing Superovulation Success." *International Embryo Transfer Society Newsletter* 9 (1): 11-17.

Armstrong, D.T., and G. Evans. 1984. "Intrauterine Insemination Enhances Fertility of Frozen Semen in Superovulated Ewes." *Journal of Reproduction and Fertility* 71: 89-94.

Armstrong, D.T., et al. 1983. "Superovulation Treatments and Embryo Transfer in Angora Goats." *Journal of Reproduction and Fertility* 67: 403-10.

Avery, B., and M. Schmidt. 1989. "Sex Determination of Bovine Embryos Using H-Y Antibodies." *Acta Veterinaria Scandinavica* 30: 155-64.

Avery, B., V. Madison, and T. Greve. 1991. "Sex and Development in Bovine In Vitro Fertilized Embryos." *Theriogenology* 35: 953-63.

Ayalon, N. 1978. "A Review of Embryonic Mortality in Cattle." *Journal of Reproduction and Fertility* 54: 483-93.

Baker, R.D. 1989. "Embryo Transfer Calves Produced in the United States from 1973 to 1988." *Proceedings of the American Embryo Transfer Association Meeting*. Nashville.

Baril, G., and J.C. Vallet. 1990. "Effects of Season on Embryo Production in Dairy Goat Hand Mated or Cervically Inseminated After Superovulatory Treatment." *Proceedings of the 6th Meeting of the European Embryo Transfer Association*. Lyon: EETA.

Barnes, F.L., C.R. Looney, and M.E. Westhusin. 1991. "Embryo Cloning in Cattle: The Current State of Technology." *Embryo Transfer* 6: 1-5.

Batt, P.A., D.K. Gardner, and A.W.N. Cameron. 1991. "Oxygen Concentration and Protein Source Affect the Development of Preimplantation Goat Embryos In Vitro." *Reproduction, Fertility, and Development* 3: 601-607.

Battye, K.M., et al. 1991. "Production of Platelet-Activating Factor by the Pre-Implantation Sheep Embryo." *Journal of Reproduction and Fertility* 93: 507-14.

Bavister, B.D. 1988. "Role of Oviductal Secretions in Embryonic Growth In Vivo and In Vitro." *Theriogenology* 29: 143-54.

Bavister, B.D., T.A. Rose-Hellekant, and T. Pinyopummintr. 1992. "Development of In Vitro Matured/In Vitro Fertilized Bovine Embryos into Morulae and Blastocysts in Defined Culture Media." *Theriogenology* 37: 127-46.

Bazer, F.W., et al. 1991. "Physiological Mechanisms of Pregnancy Recognition in Ruminants." *Journal of Reproduction and Fertility* (Suppl. 43): 39-47.

Betteridge, K.J., ed. 1977. *Embryo Transfer in Farm Animals: A Review of Techniques and Applications*. Ottawa: Department of Agriculture.

Betteridge, K.J. 1981. "An Historical Look at Embryo Transfer." *Journal of Reproduction and Fertility* 62: 1-13.

—. 1986. "Increasing Productivity in Farm Animals." In *Reproduction in Mammals. Book 5: Manipulating Reproduction*. 2d ed., ed. C.R. Austin and R.V. Short. New York: Cambridge University Press.

—. 1989a. "Livestock Embryo Sexing: Past, Present and Future." In *Evolutionary Mechanisms in Sex Determination*, ed. S.S. Wachtel. Boca Raton: CRC Press.

—. 1989b. "Structure and Function of the Equine Capsule in Relation to Embryo Manipulation and Transfer." *Equine Veterinary Journal* (Suppl. 8): 92-100.

—. 1990a. "Progress in Embryo Manipulation: Implications for the ET Industry." *Proceedings of the Annual Meeting of the Society for Theriogenology*, Toronto, August 10-11, 1990. Toronto: The Society.

—. 1990b. "Embryo Transfer in a Biotechnological Age." In *Animal Industry Vision in the 90's Through Technology*. Proceedings of the Second Congress Veterinary Association of Malaysia, 6-10 October, 1990. Kuala Lumpur: Veterinary Association Malaysia.

—. 1993. "Embryo Transfer." In *Reproduction in Domesticated Animals*, ed. G.J. King. Amsterdam: Elsevier Science.

Betteridge, K.J., and J.-E. Fléchon. 1988. "The Anatomy and Physiology of Pre-Attachment Bovine Embryos." *Theriogenology* 29: 155-87.

Betteridge, K.J., and N.M. Loskutoff. 1993. "Prospects for Improving the Survival Rate of Transferred Embryos." *Molecular Reproduction and Development* 36: 262-65.

Betteridge, K.J., and C. Smith. 1988. "Extending the Use of Embryo Transfer in Farm Animals." *Proceedings of the XIth International Congress of Animal Reproduction and Artificial Insemination* (Dublin) 5: 255-64.

Betteridge, K.J., M.D. Eaglesome, and P.F. Flood. 1979. "Embryo Transport Through the Mare's Oviduct Depends upon Cleavage and Is Independent of the Ipsilateral Corpus Luteum." *Journal of Reproduction and Fertility* (Suppl. 27): 387-94.

Betteridge, K.J., W.A. King, and D. Rieger. 1989a. "Bovine Embryo Development from Conception to Collection." *Proceedings of the American Embryo Transfer Association Meeting*. Nashville.

Betteridge, K.J., et al. 1982. "Development of Horse Embryos up to Twenty-Two Days After Ovulation: Observations on Fresh Specimens." *Journal of Anatomy* 135 (Pt. 1)(August): 191-209.

—. 1989b. "Potential Genetic Improvement of Cattle by Fertilization of Fetal Oocytes *In Vitro*." *Journal of Reproduction and Fertility* (Suppl. 38): 87-98.

Biggers, J.D. 1983. "Generation of the Human Life Cycle." In *Abortion and the Status of the Fetus*, ed. W.B. Bondeson. Dordrecht: D. Reidel.

- . 1990. "Arbitrary Partitions of Prenatal Life." *Human Reproduction* 5: 1-6.
- . 1991. "Walter Heape, FRS: A Pioneer in Reproductive Biology. Centenary of His Embryo Transfer Experiments." *Journal of Reproduction and Fertility* 93: 173-86.
- Bishop, M. 1964. "Paternal Contribution to Embryonic Death." *Journal of Reproduction and Fertility* 7: 383-96.
- Blumenfeld, Z., and B. Lunenfeld. 1989. "The Potentiating Effect of Growth Hormone on Follicle Stimulation with Human Menopausal Gonadotropin in a Panhypopituitary Patient." *Fertility and Sterility* 52: 328-31.
- Boland, M.P., D. Goulding, and J.F. Roche. 1991. "Alternative Gonadotrophins for Superovulation in Cattle." *Theriogenology* 35: 5-17.
- Bondioli, K.R. 1992. "Commercial Cloning of Cattle by Nuclear Transfer." *Proceedings of Symposium on Cloning Mammals by Nuclear Transplantation*. Fort Collins.
- Bondioli, K.R., M.E. Westhusin, and C.R. Looney. 1990. "Production of Identical Bovine Offspring by Nuclear Transfer." *Theriogenology* 33: 165-74.
- Bondioli, K.R., et al. 1989. "The Use of Male-Specific Chromosomal DNA Fragments to Determine the Sex of Bovine Preimplantation Embryos." *Theriogenology* 31: 95-104.
- Boaman, P., et al. 1989. "Sexing Bovine Embryos with Monoclonal Antibodies Against the H-Y Antigen." *Livestock Production Science* 23 (1-2): 1-16.
- Brackett, B.G., et al. 1982. "Normal Development Following *In Vitro* Fertilization in the Cow." *Biology of Reproduction* 27: 147-58.
- Briggs, R., and T.J. King. 1952. "Transplantation of Living Nuclei from Blastula Cells into Enucleated Frog Eggs." *Proceedings of the National Academy of Sciences of the United States of America* 38: 455-63.
- Broadbent, P.J., M. Stewart, and D.F. Dolman. 1991. "Recipient Management and Embryo Transfer." *Theriogenology* 35: 125-39.
- Broermann, D.M., et al. 1990. "Limitations of Oviductal Transfers in Swine." *Theriogenology* 33: 709-21.
- Bruyas, J.F., et al. 1988. "Actualités et perspectives d'avenir de la transplantation embryonnaire chez les bovins." *Revue de médecine vétérinaire* 139: 917-34.
- Buckrell, B.C., et al. 1990. "Failure to Maintain Interspecific Pregnancy After Transfer of Dall's Sheep Embryos to Domestic Ewes." *Journal of Reproduction and Fertility* 90: 387-94.
- . 1992. "A Breeding Trial Using a Transcervical Technique for Artificial Insemination in Sheep." *Proceedings of the 12th International Congress on Animal Reproduction* (The Hague) 3: 1531-33.
- Butler, J.E., and J.D. Biggers. 1989. "Assessing the Viability of Preimplantation Embryos *In Vitro*." *Theriogenology* 31: 115-26.
- Carmichael, R.A. 1980. "History of the International Embryo Transfer Society. Part I." *Theriogenology* 13: 3-6.

Carney, N.J., et al. 1991. "Three-Year Study of Pregnancy Rates Resulting from Transfer of Fresh Versus Cooled, Transported Equine Embryos." *Theriogenology* 35: 189.

Chang, M.C. 1959. "Fertilization of Rabbit Ova *In Vitro*." *Nature* 184: 466-67.

—. 1983. "My Work on the Transplantation of Mammalian Eggs." *Theriogenology* 19: 293-303.

Christensen, L.G. 1991. "Use of Embryo Transfer in Future Cattle Breeding Schemes." *Theriogenology* 35: 141-49.

Church, R.B. 1987. "Embryo Manipulation and Gene Transfer in Domestic Animals." *Trends in Biotechnology* 5 (January): 13-19.

Church, R.B., and B. Shea. 1976. "Some Aspects of Bovine Embryo Transfer." In *Egg Transfer in Cattle*, ed. L.E.A. Rowson. EUR 5491. Luxembourg: Commission of the European Communities.

Cook, V.M., et al. 1989. "Pregnancy Rates of Cooled, Transported Equine Embryos." *Equine Veterinary Journal* (Suppl. 8): 80-81.

Croy, B.A., et al. 1988. "Assessment of Immunoregulation by Cultured, Pre-Attachment Bovine Embryos." *Journal of Reproductive Immunology* 14: 9-25.

Curran, S. 1992. "Fetal Sex Determination in Cattle and Horses by Ultrasonography." *Theriogenology* 37: 17-21.

Dieleman, S.J., et al. 1989. "Improved Embryo Yield and Condition of Donor Ovaries in Cows after PMSG Superovulation with Monoclonal Anti-PMSG Administered Shortly After the Preovulatory LH Peak." *Theriogenology* 31: 473-87.

Dorrington, J.H., et al. 1987. "Actions of Growth Factors in the Follicle." *Journal of Steroid Biochemistry* 27: 405-11.

Driancourt, M.A. 1991. "Follicular Dynamics in Sheep and Cattle." *Theriogenology* 35: 56-79.

Elsden, R.P., L.D. Nelson, and G.E. Seidel, Jr. 1978. "Superovulating Cows with Follicle Stimulating Hormone and Pregnant Mare's Serum Gonadotrophin." *Theriogenology* 9: 17-26.

Enders, A.C., and I.K.M. Liu. 1991. "Lodgement of the Equine Blastocyst in the Uterus from Fixation Through Endometrial Cup Formation." *Journal of Reproduction and Fertility* (Suppl. 44): 427-38.

Eppig, J.J., and A.C. Schroeder. 1989. "Capacity of Mouse Oocytes from Preantral Follicles to Undergo Embryogenesis and Development to Live Young After Growth, Maturation, and Fertilization *In Vitro*." *Biology of Reproduction* 41: 268-76.

European Embryo Transfer Association. 1990. "National Statistic Data of the Embryo Transfer Activity for Cattle, in Europe, 1989." *Proceedings of the 6th Meeting of the European Embryo Transfer Association*. Lyon: EETA.

Evans, M.J., et al. 1990. "Derivation and Preliminary Characterization of Pluripotent Cell Lines from Porcine and Bovine Blastocysts." *Theriogenology* 33: 125-28.

Eyestone, W.H., and N.L. First. 1991. "Characterization of Developmental Arrest in Early Bovine Embryos Cultured *In Vitro*." *Theriogenology* 35: 613-24.

Fehilly, C.B., and S.M. Willadsen. 1986. "Embryo Manipulation in Farm Animals." *Oxford Reviews of Reproductive Biology* 8: 379-413.

Ferris, T.A., and B.W. Troyer. 1987. "Breakeven Costs for Embryo Transfer in a Commercial Dairy Herd." *Journal of Dairy Science* 70: 2394-2401.

Filicori, M., et al. 1991. "Ovulation Induction with Pulsatile Gonadotropin-Releasing Hormone: Technical Modalities and Clinical Perspectives." *Fertility and Sterility* 56: 1-13.

Fischer, B., et al. 1988. "Potential Risk of Light and Room Temperature Exposure to Preimplantation Embryos." *Fertility and Sterility* 50: 938-44.

Flood, P.F. 1991. "The Development of the Conceptus and Its Relationship to the Uterus." In *Reproduction in Domestic Animals*. 4th ed., ed. P.T. Cupps. New York: Academic Press.

Freeman, A.E., and D.D. Beitz. 1992. "Cytoplasmic Inheritance — Molecular Differences and Phenotypic Expression." *Proceedings of Symposium on Cloning Mammals by Nuclear Transplantation*. Fort Collins.

Frydman, R., et al. 1991. "Prevention of Premature Luteinizing Hormone and Progesterone Rise with a Gonadotropin-Releasing Hormone Antagonist, Nal-Glu, in Controlled Ovarian Hyperstimulation." *Fertility and Sterility* 56: 923-27.

Gandolfi, F., et al. 1989. "Characterization of Proteins Secreted by Sheep Oviduct Epithelial Cells and their Function in Embryonic Development." *Development* 106: 303-12.

Gardner, D.K., and P. Batt. 1991. "Nutrient Uptake by the Preimplantation Sheep Embryo." In *Australian Society for Reproductive Biology, Twenty-Third Annual Conference: University of Sydney, September 30-October 2, 1991: Programme and Abstracts of Papers*. Australia: The Society.

Gardner, D.K., and H.J. Leese. 1987. "Assessment of Embryo Viability Prior to Transfer by the Noninvasive Measurement of Glucose Uptake." *Journal of Experimental Zoology* 242: 103-105.

Georges, M. 1991. "Perspectives for Marker-Assisted Selection and Velogenetics in Animal Breeding." In *Animal Applications of Research in Mammalian Development*, ed. R.A. Pedersen, A. McLaren, and N.L. First. Plainview: Cold Spring Harbor Laboratory Press.

Georges, M., and J.M. Massey. 1991. "Velogenetics, or the Synergistic Use of Marker Assisted Selection and Germ-Line Manipulation." *Theriogenology* 35: 151-59.

Gerrity, M. 1992. "Determinants of Human Embryo Quality Following *In Vitro* Fertilization." *Theriogenology* 37: 147-60.

Gilbert, D.E., et al. 1990. "Comparison of a Progesterone Intravaginal Device (CIDRTM) with Flunixin Meglumine (FinadyneTM) for Reducing the Effects of Corpora Lutea Regression in the Goat." *Theriogenology* 33: 230.

Glasgow, L. 1989. "Kit for Sexing Embryos Sets to Work Down on the Farm." *New Scientist* 124 (9 December): 31.

Godkin, G.F. 1986. "Fertility and Twinning in Canadian Reindeer." *Rangifer*, Special Issue No. 1: 145-50.

Gong, J.G., T. Bramley, and R. Webb. 1991a. "The Effect of Recombinant Bovine Somatotropin on Ovarian Function in Heifers: Follicular Populations and Peripheral Hormones." *Biology of Reproduction* 45: 941-49.

—. 1991b. "The Effect of Recombinant Bovine Somatotropin (BST) on Ovarian Follicular Dynamics in Heifers." *Journal of Reproduction and Fertility Abstract Series* 8: 6.

Gordon, I. 1991. "Potential Application of Cattle *In Vitro* Fertilization in Commercial Practice and Research." *International Embryo Transfer Society Newsletter* 9 (2): 4-9.

Gordon, I., and K.H. Lu. 1990. "Production of Embryos *In Vitro* and Its Impact on Livestock Production." *Theriogenology* 33: 77-87.

Goto, K., et al. 1990. "Fertilisation of Bovine Oocytes by the Injection of Immobilised, Killed Spermatozoa." *Veterinary Record* 127: 517-20.

Greve, T., and H. Callesen. 1989. "Selection and Management of Donor Cattle: Improvement of Embryo Yield." *Proceedings of the 5th Meeting of the European Embryo Transfer Association*. Lyon: EETA.

Greve, T., and V. Madison. 1991. "In Vitro Fertilization in Cattle: A Review." *Reproduction, Nutrition, Development* 31: 147-57.

Guilbault, L.A., et al. 1991. "Decreased Superovulatory Responses in Heifers Superovulated in the Presence of a Dominant Follicle." *Journal of Reproduction and Fertility* 91: 81-89.

Hammond, J. 1962. "Sir John Hammond, CBE, FRS: An Interview." *Journal of Reproduction and Fertility* 3: 2-13.

Hammond, J.M., et al. 1991. "The Ovarian Insulin-Like Growth Factors, a Local Amplification Mechanism for Steroidogenesis and Hormone Action." *Journal of Steroid Biochemistry and Molecular Biology* 40: 411-16.

Handyside, A.H., et al. 1989. "Biopsy of Human Preimplantation Embryos and Sexing by DNA Amplification." *Lancet* (18 February): 347-49.

Hare, W.C.D., et al. 1976. "Sexing Two-Week-Old Bovine Embryos by Chromosomal Analysis Prior to Surgical Transfer: Preliminary Methods and Results." *Theriogenology* 5: 243-53.

Harney, J.P., and F.W. Bazer. 1989. "Effect of Porcine Conceptus Secretory Proteins on Interestrous Interval and Uterine Secretion of Prostaglandins." *Biology of Reproduction* 41: 277-84.

Hasler, J.F., et al. 1987. "Effect of Donor-Embryo-Recipient Interactions of Pregnancy Rate in a Large-Scale Bovine Embryo Transfer Program." *Theriogenology* 27: 139-68.

Hayashi, S., et al. 1989. "Birth of Piglets from Frozen Embryos." *Veterinary Record* 125: 43-44.

Heape, W. 1891. "Preliminary Note on the Transplantation and Growth of Mammalian Ova Within a Uterine Foster Mother." *Proceedings of the Royal Society of London* 48: 457-58.

Herr, C.M., et al. 1990. "Sex of Progeny from Bovine Embryos Sexed with a Rapid Y-Chromosome-Detection Assay." *Theriogenology* 33: 247.

Herrler, A., E. Farries, and H. Niemann. 1990. "A Trial to Stimulate Insulin Like Growth Factor Levels to Improve Superovulatory Response in Dairy Cows." *Theriogenology* 33: 248.

Heyman, Y., and Y. Ménézo. 1987. "Interaction of Trophoblastic Vesicles with Bovine Embryos Developing *In Vitro*." In *The Mammalian Preimplantation Embryo. Regulation of Growth and Differentiation *In Vitro**, ed. B.D. Bavister. New York: Plenum Press.

Hinrichs, K., and L.M. DiGiorgio. 1991. "Embryonic Development After Intra-Follicular Transfer of Equine Oocytes." *Journal of Reproduction and Fertility* (Suppl. 44): 369-94.

Hinrichs, K., et al. 1985. "Pregnancy in Ovariectomised Mares Achieved by Embryo Transfer: A Preliminary Study." *Equine Veterinary Journal* (Suppl. 3): 74-75.

Hockley, D.K., et al. 1992. "Superovulation with a Single Subcutaneous Injection of Folltropin in the Cow: Effect of Dose and Site of Injection." *Theriogenology* 37: 224.

Holstein World. 1991. (October).

Homburg, R., et al. 1990. "Cotreatment with Human Growth Hormone and Gonadotropins for Induction of Ovulation: A Controlled Clinical Trial." *Fertility and Sterility* 53: 254-60.

Howard, J.C. 1991. "Disease and Evolution." *Nature* 352: 565-67.

Human Reproduction. 1991. Vol. 6 (1).

Hunter, R.H.F., B. Fléchon, and J.E. Fléchon. 1991. "Distribution, Morphology and Epithelial Interactions of Bovine Spermatozoa in the Oviduct Before and After Ovulation: A Scanning Electron Microscope Study." *Tissue and Cell* 23: 641-56.

Huppert, L.C. 1979. "Induction of Ovulation with Clomiphene Citrate." *Fertility and Sterility* 31: 1-8.

Ibrahim, Z.H.Z., et al. 1991. "The Use of Biosynthetic Human Growth Hormone to Augment Ovulation Induction with Buserelin Acetate/Human Menopausal Gonadotropin in Women with a Poor Ovarian Response." *Fertility and Sterility* 55: 202-204.

International Embryo Transfer Society. 1989. "Conclusions de la Société internationale de Transfert d'Embryons (IETS)." *Revue scientifique et technique* (International Office of Epizootics) 8: 569-70.

Johnson, L.A. 1992. "Advances in Sex Pre-Determination Using Flow Cytometrically Sorted X- and Y-Chromosomes Bearing Sperm." *International Embryo Transfer Society Newsletter* 10 (2): 5-11.

Johnson, L.A., J.P. Flook, and H.W. Hawk. 1989. "Sex Preselection in Rabbits: Live Births from X and Y Sperm Separated by DNA and Cell Sorting." *Biology of Reproduction* 41: 199-203.

Jubb, T., et al. 1990. "Failure of an Intramuscular Injection of an Analogue of Gonadotropin-Releasing Hormone 11 to 13 Days After Insemination to Increase Pregnancy Rates in Dairy Cattle." *Australian Veterinary Journal* 67: 359-61.

Kaaekuahiwi, M.A., and A.R. Menino, Jr. 1990. "Relationship Between Plasminogen Activator Production and Bovine Embryo Development *In Vitro*." *Journal of Animal Science* 68: 2009-14.

Kashiwazaki, N., et al. 1991. "Production of Normal Piglets from Hatched Blastocysts Frozen at -196°C." *Veterinary Record* 128: 256-57.

King, W.A. 1990. "Chromosome Abnormalities and Pregnancy Failure in Domestic Animals." In *Advances in Veterinary Science and Comparative Medicine* 34: 229-50.

King, W.A., et al. 1979. "A Method for Preparation of Chromosomes from Bovine Zygotes and Blastocysts." *Veterinary Science Communications* 3 (July): 51-56.

Kirkwood, R.N., et al. 1988. "The Influence of Exogenous Growth Hormone on Ovulation Rate in Gilts." *Canadian Journal of Animal Science* 68: 1097-1103.

—. 1990. "Observations on the Influence of Active Immunization Against Somatostatin on the Reproductive Performance of Sheep and Pigs." *Journal of Reproductive Immunology* 17: 229-38.

Knight, C.H., et al. 1988. "Exogenous GnRH Induces Ovulation in Seasonally Anoestrous Lactating Goats (*Capra hircus*)."*Journal of Reproduction and Fertility* 83: 679-86.

Kohen, G., et al. 1990. "Sexage rapide des embryons bovins par amplification d'ADN." *Proceedings of the 6th Meeting of the European Embryo Transfer Association*. Lyon: EETA.

Kruip, T.A.M., et al. 1991. "A New Method for Bovine Embryo Production: A Potential Alternative to Superovulation." *Veterinary Record* 128: 208-10.

Lajili, H., P. Humblot, and M. Thiblier. 1991. "Effect of PGF2 Alpha Treatment on Conception Rates of Dairy Cows Treated with a GnRH Agonist 12 to 14 Days After Artificial Insemination." *Theriogenology* 36: 335-47.

Lambert, R.D., et al. 1986. "In Vitro Fertilization of Bovine Oocytes Matured *In Vivo* and Collected at Laparoscopy." *Theriogenology* 25: 117-33.

Larson, R.C., G.G. Ignatz, and W.B. Currie. 1992. "Platelet Derived Growth Factor (PDGF) Stimulates Development of Bovine Embryos During the Fourth Cell Cycle." *Development* 115: 821-26.

Lawitts, J.A., and J.D. Biggers. 1991. "Optimization of Mouse Embryo Culture Media Using Simplex Methods." *Journal of Reproduction and Fertility* 91: 543-56.

Leese, H.J. 1990. "Energy Metabolism of the Preimplantation Embryo." In *Early Embryo Development and Paracrine Relationships*, ed. S. Heyner and L.M. Wiley. New York: Alan R. Liss.

—. 1991. "Metabolism of the Preimplantation Mammalian Embryo." *Oxford Reviews of Reproductive Biology* 13: 35-72.

Leese, H.J., and A.M. Barton. 1984. "Pyruvate and Glucose Uptake by Mouse Ova and Preimplantation Embryos." *Journal of Reproduction and Fertility* 72: 9-13.

Lehn-Jensen, H. 1986. "Cryopreservation of Bovine Embryos: An Evaluation of Factors Influencing the Survival of Day 6 1/2-7 1/2 Embryos During Freezing

and Thawing." Dissertation, Royal Veterinary and Agricultural University, Institute of Animal Reproduction, Copenhagen.

Leibo, S.P. 1986. "Cryobiology: Preservation of Mammalian Embryos." In *Genetic Engineering of Animals: An Agricultural Perspective*, ed. J.W. Evans and A. Hollaender. New York: Plenum Press.

- . 1989. "Equilibrium and Non-Equilibrium Cryopreservation of Embryos." *Theriogenology* 31: 85-93.
- . 1990. "The Basics of Cryopreservation of Bovine Embryos." *Proceedings of the American Embryo Transfer Association Meeting*. Madison.

Leibo, S.P., and W.F. Rall. 1990. "Prenatal Diagnosis of Sex in Bovine Fetuses by Amniocentesis." *Theriogenology* 33: 531-52.

Leibo, S.P., and D. Winninger. 1986. "Production of Bovine Pregnancies from Embryos Transported at 0°C by Air." *Theriogenology* 25: 165.

Lindner, G.M., and R.W. Wright, Jr. 1983. "Bovine Embryo Morphology and Evaluation." *Theriogenology* 20: 407-16.

Loskutoff, N.M. 1990. "Micromanipulation of Embryos and Gametes." *International Embryo Transfer Society Newsletter* 8 (3): 5-14.

Loskutoff, N.M., W.H. Johnson, and K.J. Betteridge. 1993. "The Developmental Competence of Bovine Embryos with Reduced Cell Numbers." *Theriogenology* 39: 95-107.

Loskutoff, N.M., et al. 1992. "Viability of Biopsied, Monozygotic Twin Embryos Produced from 4-Cell, 8-Cell and Post-Compaction Stage Bovine Embryos Generated In Vitro." *Theriogenology* 37: 251.

Loumaye, E. 1990. "The Control of Endogenous Secretion of LH by Gonadotropin-Releasing Hormone Agonists During Ovarian Hyperstimulation for In-Vitro Fertilization and Embryo Transfer." *Human Reproduction* 5: 357-76.

McGuirk, B. 1989. "The Relevance of MOET Programmes to Developing Countries." *Theriogenology* 31: 29-40.

McKelvey, W.A.C., et al. 1986. "Repeated Recoveries of Embryos from Ewes by Laparoscopy." *Theriogenology* 25: 855-65.

MacLennan, D.H., et al. 1990. "Ryanodine Receptor Gene is a Candidate for Predisposition to Malignant Hyperthermia." *Nature* 343: 559-61.

McLeod, B.J., et al. 1991. "Efficacy of Intermittent or Continuous Administration of GnRH in Inducing Ovulation in Early and Late Seasonal Anoestrus in the Père David's Deer Hind (*Elaphurus davidianus*)." *Journal of Reproduction and Fertility* 91: 229-38.

Macmillan, K.L., et al. 1991. "Effects of Supplemental Progesterone on Pregnancy Rates in Cattle." *Journal of Reproduction and Fertility* (Suppl. 43): 304.

McNatty, K.P., et al. 1989. "Superovulation and Embryo Recovery in Goats Treated with Ovagen and Folltropin." *New Zealand Veterinary Journal* 37: 27-29.

McNeilly, A.S., and H.M. Fraser. 1987. "Effect of Gonadotrophin-Releasing Hormone Agonist-Induced Suppression of LH and FSH on Follicle Growth and Corpus Luteum Function in the Ewe." *Journal of Endocrinology* 115: 273-82.

McNeilly, A.S., M. O'Connell, and D.T. Baird. 1982. "Induction of Ovulation and Normal Luteal Function by Pulsed Injections of Luteinizing Hormone in Anestrous Ewes." *Endocrinology* 110: 1292-99.

Maddox, J. 1992. "Warning on Population Growth." *Nature* 355: 759.

Madill, S. 1992. "The Use of GnRH Analogues to Delay the LH Surge and Ovulation in Superovulated Holstein Heifers." D.V.Sc. dissertation, University of Guelph.

Mapletoft, R.J. 1984. "Embryo Transfer Technology for the Enhancement of Animal Reproduction." *Biotechnology* 2: 149-60.

Mapletoft, R.J., G. Bo, and B.D. Murphy. 1991. "The Effect of Biological Activity of Gonadotropins on Superovulation in the Cow." *Revista Brasileira de Reproducao Animal* 3 (Suppl.): 74-92.

Markette, K.L., G.E. Seidel, Jr., and R.P. Elsden. 1985. "Estimation of Embryonic Losses in Bovine Embryo Transfer Recipients from Progesterone Profiles and Returns to Estrus." *Theriogenology* 23: 45-62.

Massip, A., P. Van der Zwalm, and F. Ectors. 1987. "Recent Progress in Cryopreservation of Cattle Embryos." *Theriogenology* 27: 69-79.

Massip, A., et al. 1989. "Some Significant Steps in the Cryopreservation of Mammalian Embryos with a Note on a Vitrification Procedure." *Animal Reproduction Science* 19: 117-29.

Maurer, R.R., and J.R. Chenault. 1983. "Fertilization Failure and Embryonic Mortality in Parous and Nonparous Beef Cattle." *Journal of Animal Science* 56: 1186-89.

Mazur, P. 1990. "Equilibrium, Quasi-Equilibrium and Nonequilibrium Freezing of Mammalian Embryos." *Cell Biophysics* 17: 53-92.

Meinecke, B., and S. Meinecke-Tillman. 1990. "Assessment of the Embryo Quality in Cattle and Small Ruminants." *Proceedings of the 6th Meeting of the European Embryo Transfer Association*. Lyon: EETA.

Menashe, Y., et al. 1990. "Can Growth Hormone Increase, After Clonidine Administration, Predict the Dose of Human Menopausal Hormone Needed for Induction of Ovulation?" *Fertility and Sterility* 53: 432-35.

Ménézo, Y. 1976. "Milieu synthétique pour la survie et la maturation des gamètes et pour la culture de l'oeuf fécondé." *Comptes rendus hebdomadaires des séances de l'académie des sciences. D: Sciences naturelles* 22 (282): 1967-70.

Ménézo, Y.J.R., J.-F. Guérin, and J.-C. Czyba. 1990. "Improvement of Human Early Embryo Development *In Vitro* by Coculture on Monolayers of Vero Cells." *Biology of Reproduction* 42: 301-306.

Mittwoch, U. 1989. "Sex Differentiation in Mammals and Tempo of Growth: Probabilities vs. Switches." *Journal of Theoretical Biology* 137: 445-55.

Monk, M.J., and A.H. Handyside. 1988. "Sexing of Preimplantation Mouse Embryos by Measurement of X-Linked Gene Dosage in a Single Blastomere." *Journal of Reproduction and Fertility* 82: 365-68.

Monniaux, D., D. Chupin, and J. Saumande. 1983. "Superovulatory Responses of Cattle." *Theriogenology* 19: 55-81.

Moor, R.M., T.A.M. Kruip, and D. Green. 1984. "Intraovarian Control of Folliculogenesis: Limits to Superovulation?" *Theriogenology* 21: 103-16.

Murphy, B.D., and S.D. Martinuk. 1991. "Equine Chorionic Gonadotropin." *Endocrine Reviews* 12: 27-44.

Murphy, B.D., et al. 1984. "Variability in Gonadotropin Preparations as a Factor in the Superovulatory Response." *Theriogenology* 21: 117-25.

Nagy, A., et al. 1990. "Embryonic Stem Cells Alone Are Able to Support Fetal Development in the Mouse." *Development* 110: 815-21.

Nancarrow, C.D., et al. 1991. "Expression and Physiology of Performance Regulating Genes in Transgenic Sheep." *Journal of Reproduction and Fertility* (Suppl. 43): 277-91.

Nephew, K.P., et al. 1990. "Effects of Intramuscular Administration of Recombinant Bovine Interferon-Alpha-I During the Period of Maternal Recognition of Pregnancy." *Journal of Animal Science* 68: 2766-70.

—. 1991. "Relationship Between Variation in Conceptus Development and Differences in Estrous Cycle Duration in Ewes." *Biology of Reproduction* 44: 536-39.

Newcomb, R., and L.E.A. Rowson. 1975. "Conception Rate After Uterine Transfer of Cow Eggs, in Relation to Synchronization of Estrus and Age of Eggs." *Journal of Reproduction and Fertility* 43: 539-41.

Niemann, H. 1991. "Cryopreservation of Ova and Embryos from Livestock: Current Status and Research Needs." *Theriogenology* 35: 109-24.

Olson, P., et al. 1992. "Cryopreserved Semen Extenders Can Induce Nuclear Activation and/or Cytoplasmic Fragmentation of Bovine Ova Matured *In Vitro*." *Theriogenology* 37: 268.

Overstrom, E.W., et al. 1989. "Blastocyst Oxidative Metabolism and Embryo Viability." *Journal of Cell Biology* 107: 607.

—. 1992. "Viability and Oxidative Metabolism of the Bovine Blastocyst." *Theriogenology* 37: 269.

Palmiter, R.D., et al. 1982. "Dramatic Growth of Mice That Develop from Eggs Microinjected with Metallothionein-Growth Hormone Fusion Genes." *Nature* 300: 611-15.

Parks, J.E., and N.A. Ruffing. 1992. "Factors Affecting Low Temperature Survival of Mammalian Oocytes." *Theriogenology* 37: 59-73.

Parr, R.A., et al. 1986. "An Interaction Between Nutrition and Progesterone Reduces Embryonic Survival in Sheep." In *Australian Society for Reproductive Biology, Eighteenth Annual Conference: Brisbane, Australia, 31 August-3 September, 1986: Programme and Abstracts of Papers*. Australia: The Society.

Pashen, R.L. 1987. "Short-Term Storage and Survival of Horse Embryos After Refrigeration at 4°C." *Journal of Reproduction and Fertility* (Suppl. 35): 697-98.

Picard, L., and K.J. Betteridge. 1989. "The Micromanipulation of Farm Animal Embryos." In *Animal Biotechnology: Comprehensive Biotechnology, First Supplement, Volume I*, ed. L.A. Babiuk and J.P. Phillips. Oxford: Pergamon.

Picard, L., W.A. King, and K.J. Betteridge. 1985. "Production of Sexed Calves from Frozen-Thawed Embryos." *Veterinary Record* 117: 603-608.

Picard, L., et al. 1990. "Production of Chimaeric Bovine Embryos and Calves by Aggregation of Inner Cell Masses with Morulae." *Molecular Reproduction and Development* 27: 295-304.

Pichavant, P., et al. 1990. "Taux de gestation après réfrigération et stockage des embryons bovins à 0°C." *Proceedings of the 6th Meeting of the European Embryo Transfer Association*. Lyon: EETA.

Pieterse, M.C., et al. 1988. "Aspiration of Bovine Oocytes During Transvaginal Ultrasound Scanning of the Ovaries." *Therogenology* 30: 751-62.

Pinyopummintr, T., and B.D. Bavister. 1991. "In Vitro-Matured/In Vitro-Fertilized Bovine Oocytes Can Develop into Morulae/Blastocysts in Chemically Defined, Protein-Free Culture Media." *Biology of Reproduction* 45: 736-42.

Polge, C. 1985. "How Does Embryo Manipulation Fit into Present and Future Pig Reproduction?" *Journal of Reproduction and Fertility* (Suppl. 33): 93-100.

Pollard, J.W. 1992. "Functional Biology of Bovine Oviductal Epithelial Cells In Vitro." Ph.D. dissertation, University of Guelph.

Pollard, J.W., et al. 1991. "Fertilizing Capacity of Bovine Sperm May Be Maintained by Binding of Oviductal Epithelial Cells." *Biology of Reproduction* 44: 102-107.

Pope, W.F. 1988. "Uterine Asynchrony: A Cause of Embryonic Loss." *Biology of Reproduction* 39: 990-1003.

—. 1989. "Swine Embryo Transfer — Embryonic Mortality and Degeneration." *Proceedings of the American Embryo Transfer Association Meeting*. Nashville.

Potts, W.K., C.J. Manning, and E.K. Wakeland. 1991. "Mating Patterns in Semi-natural Populations of Mice Influenced by MHC Genotype." *Nature* 352: 619-21.

Prather, R.S., and N.L. First. 1990. "Nuclear Transfer in Mammalian Embryos." *International Review of Cytology* 120: 169-90.

Pursel, V.G., et al. 1990. "Integration, Expression and Germ-Line Transmission of Growth-Related Genes in Pigs." *Journal of Reproduction and Fertility* (Suppl. 41): 77-87.

Rall, W.F. 1987. "Factors Affecting the Survival of Mouse Embryos Cryopreserved by Vitrification." *Cryobiology* 24: 387-402.

Reed, M.L., M.J. Illera, and R.M. Petters. 1992. "In Vitro Culture of Pig Embryos." *Therogenology* 37: 95-109.

Renard, J.-P. 1984. "Methods of Conserving Gametes and Embryos of Farm Mammals." *Livestock Production Science* 11: 49-59.

—. 1990. "State of the Art, Limitations and Prospective of Cloning in Domestic Animals." *Proceedings of the 6th Meeting of the European Embryo Transfer Association*. Lyon: EETA.

Renard, J.P., A. Philippon, and Y. Ménézo. 1980. "In-Vitro Uptake of Glucose by Bovine Blastocysts." *Journal of Reproduction and Fertility* 58: 161-64.

Reproduction in Domestic Animals. 1991. Vol. 26 (2).

Rexroad, C.E. 1989. "Co-Culture of Domestic Animal Embryos." *Theriogenology* 31: 105-14.

Rexroad, C.E., Jr., et al. 1990. "Insertion, Expression and Physiology of Growth-Regulating Genes in Ruminants." *Journal of Reproduction and Fertility* (Suppl. 41): 119-24.

Rieger, D. 1984. "The Measurement of Metabolic Activity as an Approach to Evaluating Viability and Diagnosing Sex in Early Embryos." *Theriogenology* 21: 138-49.

—. 1992. "Relationships Between Energy Metabolism and Development of Early Mammalian Embryos." *Theriogenology* 37: 75-93.

Rieger, D., and K.J. Betteridge. 1989. "Principles and Practice of Embryo Culture." *Proceedings of the American Embryo Transfer Association Meeting*. Nashville.

Rieger, D., D. Desaulnier, and A.K. Goff. 1988. "Ovulatory Response and Embryo Yield in Superovulated Holstein Heifers Given a Priming Dose of FSH-P at Day 2 of the Estrous Cycle." *Theriogenology* 30: 695-99.

Rieger, D., N.M. Loskutoff, and K.J. Betteridge. 1992. "Developmentally Related Changes in the Metabolism of Glucose and Glutamine by Cattle Embryos Produced and Co-Cultured *In Vitro*." *Journal of Reproduction and Fertility* 95: 585-95.

Rieger, D., et al. 1991a. "The Effect of Co-Treatment with Recombinant Bovine Somatotrophin on Plasma Progesterone Concentration and Number of Embryos Collected from Superovulated Holstein Heifers." *Theriogenology* 35: 863-68.

—. 1991b. "The Effect of Cryopreservation on the Metabolic Activity of Day-6.5 Horse Embryos." *Journal of Reproduction and Fertility* (Suppl. 44): 411-17.

Roberts, R.M. 1991. "A Role for Interferons in Early Pregnancy." *Bioessays* 13: 121-26.

Roberts, R.M., et al. 1990. "Maternal Recognition of Pregnancy and Embryonic Loss." *Theriogenology* 33: 175-83.

Robertson, E.J. 1991. "Using Embryonic Stem Cells to Introduce Mutations into the Mouse Germ Line." *Biology of Reproduction* 44: 238-45.

Robl, J.M., and S.L. Stice. 1989. "Prospects for the Commercial Cloning of Animals by Nuclear Transplantation." *Theriogenology* 31: 75-84.

Roche, J.F., and M.P. Boland. 1991. "Turnover of Dominant Follicles in Cattle of Different Reproductive States." *Theriogenology* 35: 81-90.

Romagnano, A., et al. 1987. "Analysis of X-Chromosome Inactivation in Horse Embryos." *Journal of Reproduction and Fertility* (Suppl. 35): 353-61.

Rowson, L.E.A., ed. 1976. *Egg Transfer in Cattle*. EUR 5491. Luxembourg: Commission of the European Communities.

Rowson, L.E.A., R.M. Moor, and R.A.S. Lawson. 1969. "Fertility Following Egg Transfer in the Cow: Effect of Method, Medium and Synchronization of Oestrus." *Journal of Reproduction and Fertility* 18: 517-23.

Ruane, J., and C. Smith. 1989. "The Genetic Response Possible in Dairy Cattle Improvement by Setting Up a Multiple Ovulation and Embryo Transfer (MOET) Nucleus Scheme." *Génétique, Sélection, Évolution* 21: 169-83.

Ryan, D.P., et al. 1991. "Pregnancy Rates in Dairy Cows Following the Administration of a GnRH Analogue at the Time of Artificial Insemination or at Mid-Cycle Post Insemination." *Theriogenology* 36: 367-77.

Schneider, U., and P. Mazur. 1984. "Osmotic Consequences of Cryoprotectant Permeability and Its Relation to the Survival of Frozen-Thawed Embryos." *Theriogenology* 21: 68-79.

Schultz, G.A., and S. Heyner. 1993. "Growth Factors in Preimplantation Mammalian Embryos." *Oxford Reviews of Reproductive Biology* (Forthcoming).

Science Council of Canada. 1991. "It's Everybody's Business: Submissions to the Science Council's Committee on Sustainable Agriculture." Ottawa: Science Council of Canada.

Seidel, G.E. 1991. "Embryo Transfer: The Next 100 Years." *Theriogenology* 35: 171-80.

Seidel, G.E., et al. 1989. "Cryopreservation of Equine Embryos in 1,2 Propanediol." *Equine Veterinary Journal* (Suppl. 8): 87-88.

Shea, B.F. 1981. "Evaluating the Bovine Embryo." *Theriogenology* 15: 31-42.

Shea, B.F., et al. 1976. "The Transfer of Bovine Embryos." In *Egg Transfer in Cattle*, ed. L.E.A. Rowson. EUR 5491. Luxembourg: Commission of the European Communities.

Shemesh, M., and B. Weir, eds. 1989. "Maternal Recognition of Pregnancy and Maintenance of the Corpus Luteum." *Journal of Reproduction and Fertility* (Suppl. 37).

Singh, E.L. 1987a. "Potential of Embryo Transfer to Control Transmission of Disease: A Review of Current Research." In *IETS Manual*. 2d ed. Champaign: International Embryo Transfer Society.

—. 1987b. "Recommendations for the Sanitary Handling of Embryos." In *IETS Manual*. 2d ed. Champaign: International Embryo Transfer Society.

Sirard, M.A. 1989. "Practical Aspects of In-Vitro Fertilization in Cattle." *Journal of Reproduction and Fertility* (Suppl. 38): 127-34.

Sirard, M.A., et al. 1985. "The Effects of Repeated Laparoscopic Surgery Used for Ovarian Examination and Follicular Aspiration in Cows." *Animal Reproduction Science* 9: 25-30.

Sirois, J., K.J. Betteridge, and A. Brault. 1987. "Transcervical Embryo Transfer in Horses: An Application in an Equestrian Teaching Centre." *Canadian Veterinary Journal* 28: 750-53.

Sirois, J., T.L. Kimmich, and J.E. Fortune. 1992. "FSH Injections Early in the Cycle Induce Double Ovulations in Mares." *Theriogenology* 37: 300.

Slade, N.P., et al. 1985. "A New Procedure for the Cryopreservation of Equine Embryos." *Theriogenology* 24: 45-58.

Smith, C. 1986. "Use of Embryo Transfer in Genetic Improvement of Sheep." *Animal Production* 42: 81-88.

—. 1988. "Applications of Embryo Transfer in Animal Breeding." *Theriogenology* 29: 203-12.

—. 1989. "Cloning and Genetic Improvement of Beef Cattle." *Animal Production* 49: 49-62.

Smith, L.C. 1988. "Superovulation in Sheep." *Compendium on Continuing Education for the Practicing Veterinarian* 10: 1415-24.

Smith, L.C., and I. Wilmut. 1990. "Factors Affecting the Viability of Nuclear Transplanted Embryos." *Theriogenology* 33: 153-64.

Squires, E.L. 1989. "Commercial Equine Embryo Transfer: Synopsis of Panel Discussion." *Equine Veterinary Journal* (Suppl. 8): 75.

Squires, E.L., G.E. Seidel, Jr., and A.O. McKinnon. 1989. "Transfer of Cryopreserved Equine Embryos to Progestin-Treated Ovariectomised Mares." *Equine Veterinary Journal* (Suppl. 8): 89-91.

Sreenan, J.M., and M.G. Diskin, eds. 1986. *Embryonic Mortality in Farm Animals: A Seminar in the CEC Programme of Coordination of Research on Livestock Productivity and Management*. Boston: M. Nijhoff for the Commission of the European Communities.

Sreenan, J.M., and M.G. Diskin. 1987. "Factors Affecting Pregnancy Rate Following Embryo Transfer in the Cow." *Theriogenology* 27: 99-113.

Steptoe, P.C., and R.G. Edwards. 1978. "Birth After the Reimplantation of a Human Embryo." *Lancet* (12 August): 366.

Stice, S.L. 1992. "Multiple Generation Bovine Embryo Cloning." *Proceedings of Symposium on Cloning Mammals by Nuclear Transplantation*. Fort Collins.

Stringfellow, D.A., and S.M. Seidel, eds. 1990. *Manual of the International Embryo Transfer Society: A Procedural Guide and General Information for the Use of Embryo Transfer Technology, Emphasizing Sanitary Precautions*. 2d ed. Champaign: International Embryo Transfer Society.

Taylor, C.S., A.J. Moore, and R.B. Thiessen. 1986. "Single Sex Beef Cattle Systems." In *Exploiting New Technologies in Animal Breeding: Genetic Developments*, ed. C. Smith, J.W.B. King, and J.C. McKay. New York: Oxford University Press.

Taylor, C.S., et al. 1985. "Efficiency of Food Utilization in Traditional and Sex-Controlled Systems of Beef Production." *Animal Production* 40: 401-40.

Telford, N.A., A.J. Watson, and G.A. Schultz. 1990. "Transition from Maternal to Embryonic Control in Early Mammalian Development: A Comparison of Several Species." *Molecular Reproduction and Development* 26: 90-100.

Tervit, H.R. 1989. "Embryo Transfer and Sperm Sexing." In *International Congress for Sheep Veterinarians, Sheep and Beef Cattle Society of New Zealand, Organisers and Hosts to the Second International Congress for Sheep Veterinarians: February 12-16th, 1989, Massey University, Palmerston North, New Zealand*. Wellington: The Association.

Thatcher, W.W., et al. 1993. "New Clinical Uses of GnRH and Its Analogues in Cattle." *Animal Reproduction Science* 33: 27-49.

Thibier, M. 1990. "Le transfert embryonnaire: le moyen le plus sûr, au plan sanitaire, d'échanges de gènes." *Proceedings of the 6th Meeting of the European Embryo Transfer Association*. Lyon: EETA.

Thompson, J.G.E., et al. 1989. "Development of Sheep Preimplantation Embryos in Media Supplemented with Glucose and Acetate." *Theriogenology* 32: 323-30.

—. 1990. "Effect of Oxygen Concentration on In-Vitro Development of Preimplantation Sheep and Cattle Embryos." *Journal of Reproduction and Fertility* 89: 573-78.

—. 1992. "Requirement for Glucose During In Vitro Culture of Sheep Preimplantation Embryos." *Molecular Reproduction and Development* 31: 253-57.

Tiffin, G.J., et al. 1991. "Glucose and Glutamine Metabolism in Pre-Attachment Cattle Embryos in Relation to Sex and Stage of Development." *Journal of Reproduction and Fertility* 93: 125-32.

Tsunoda, Y., T. Tokunaga, and T. Sugie. 1985. "Altered Sex Ratio of Live Young After Transfer of Fast- and Slow-Developing Mouse Embryos." *Gamete Research* 12: 301-304.

Turner, K., et al. 1991. "Measurement of Pyruvate Uptake by Human Embryos in the Natural Cycle Using a Non-Invasive Technique." *Serono Symposium on Preimplantation Embryo Development*, vol. 48.

Utsumi, K., E. Satoh, and A. Iritani. 1991. "Sexing of Rat Embryos with Antisera Specific for Male Rats." *Journal of Experimental Zoology* 260: 99-105.

Utsumi, K., E. Satoh, and M. Yuhara. 1984. "Sexing of Goat and Cow Embryos by Rat H-Y Antibody." *Proceedings of the Xth International Congress on Animal Reproduction and Artificial Insemination* (Urbana) 2: 234-36.

Van Cleeff, J., M. Drost, and W.W. Thatcher. 1991. "Effects of Postinsemination Progesterone Supplementation on Fertility and Subsequent Estrous Responses of Dairy Heifers." *Theriogenology* 36: 795-807.

Vanderhyden, B.C., et al. 1990. "Developmental Pattern of the Secretion of Cumulus Expansion-Enabling Factor by Mouse Oocytes and the Role of Oocytes in Promoting Granulosa Cell Differentiation." *Developmental Biology* 140: 307-17.

van Vliet, R.A., A.M.V. Gibbins, and J.S. Walton. 1989. "Livestock Embryo Sexing: A Review of Current Methods, with Emphasis on Y-Specific DNA Probes." *Theriogenology* 32: 421-38.

Villahoz, M.D., et al. 1985. "Some Observations on Early Embryonic Death in Mares." *Theriogenology* 23: 915-24.

Walker, S.K., T.M. Heard, and R.F. Seemark. 1992. "In Vitro Culture of Sheep Embryos Without Co-Culture: Successes and Perspectives." *Theriogenology* 37: 111-26.

Walker, S.K., et al. 1989. "The Use of Synthetic Gonadotropin Releasing Hormone Treatment in the Collection of Sheep Embryos." *Theriogenology* 31: 741-52.

Walters, D.L., et al. 1982. "Pituitary and Ovarian Function in Postpartum Beef Cows. III. Induction of Estrus, Ovulation and Luteal Function with

Intermittent Small-Dose Injections of GnRH." *Biology of Reproduction* 26: 655-62.

Warwick, B.L., and R.O. Berry. 1949. "Inter-Generic and Intra-Specific Embryo Transfers in Sheep and Goats." *Journal of Heredity* 40: 297-303.

Weber, J.A., et al. 1991. "Prostaglandin E2 Secretion by Oviductal Transport-Stage Equine Embryos." *Biology of Reproduction* 45: 540-43.

Westhusin, M.E., J.H. Pryor, and K.R. Bondioli. 1991. "Nuclear Transfer in the Bovine Embryo: A Comparison of 5-Day, 6-Day, Frozen-Thawed, and Nuclear Transfer Donor Embryos." *Molecular Reproduction and Development* 28: 119-23.

White, K.L., G.B. Anderson, and R.H. Bondurant. 1987. "Expression of a Male-Specific Factor on Various Stages of Preimplantation Bovine Embryos." *Biology of Reproduction* 37: 867-73.

Whittingham, D.G., S.P. Leibo, and P. Mazur. 1972. "Survival of Mouse Embryos Frozen to -196° and -269°C ." *Science* 178: 411-14.

Wildt, D.E., et al. 1992. "Embryogenesis in Conservation Biology — or How to Make an Endangered Species Embryo." *Theriogenology* 37: 161-84.

Willadsen, S.M. 1986. "Nuclear Transplantation in Sheep Embryos." *Nature* 320: 63-65.

—. 1989. "Cloning of Sheep and Cow Embryos." *Genome* 31: 956-62.

Willadsen, S.M., et al. 1991. "The Viability of Late Morulae and Blastocysts Produced by Nuclear Transplantation in Cattle." *Theriogenology* 35: 161-70.

Willett, E.L., et al. 1951. "Successful Transplantation of a Fertilized Bovine Ovum." *Science* 113: 247.

Williams, T.J. 1986. "A Technique for Sexing Mouse Embryos by a Visual Colorimetric Assay of the X-Linked Enzyme, Glucose 6-Phosphate Dehydrogenase." *Theriogenology* 25: 733-39.

Wilmut, I. 1972. "The Effect of Cooling Rate, Warming Rate, Cryoprotective Agent, and Stage of Development on Survival of Mouse Embryos During Freezing and Thawing." *Life Sciences* 11: 1071-79.

Wilmut, I., and L.E.A. Rowson. 1973. "Experiments on the Low-Temperature Preservation of Cow Embryos." *Veterinary Record* 92: 686-90.

Wilmut, I., K.H.S. Campbell, and G.T. O'Neill. 1992a. "Sources of Totipotent Nuclei Including Embryonic Stem Cells." *Proceedings of Symposium on Cloning Mammals by Nuclear Transplantation*. Fort Collins.

Wilmut, I., C.S. Haley, and J.A. Wooliams. 1992b. "Impact of Biotechnology on Animal Breeding." *Animal Reproduction Science* 28: 149-62.

Wilmut, I., M.L. Hooper, and J.P. Simons. 1991. "Genetic Manipulation of Mammals and Its Application in Reproductive Biology." *Journal of Reproduction and Fertility* 92: 245-79.

Wilmut, I., et al. 1990. "Methods of Gene Transfer and Their Potential Use to Modify Milk Composition." *Theriogenology* 33: 113-23.

Wilson, J.M., et al. 1989. "Recombinant Bovine Follicle-Stimulating Hormone: Dose and Duration Regimens for Superovulation of Embryo Donors." *Theriogenology* 31: 273.

Wright, R.W., Jr., and K.R. Bondioli. 1981. "Aspects of *In Vitro* Fertilization and Embryo Culture in Domestic Animals." *Journal of Animal Science* 53: 702-29.

Xu, K.P., B. Hill, and K.J. Betteridge. 1992a. "Application of *In-Vitro* Fertilization Techniques to Obtain Calves from Valuable Cows After Slaughter." *Veterinary Record* 130: 204-206.

Xu, K.P., et al. 1992b. "Development and Viability of Bovine Embryos Derived from Oocytes Matured and Fertilized *In Vitro* and Co-Cultured with Bovine Oviducal Epithelial Cells." *Journal of Reproduction and Fertility* 94: 33-43.

—. 1992c. "Sex-Related Differences in Developmental Rates of Bovine Embryos Produced and Cultured *In Vitro*." *Molecular Reproduction and Development* 31: 249-52.

Zavy, M.T., et al. 1982. "Identification of Stage-Specific and Hormonally Induced Polypeptides in the Uterine Protein Secretions of the Mare During the Oestrous Cycle and Pregnancy." *Journal of Reproduction and Fertility* 64: 199-207.

Human Embryo Research: Past, Present, and Future

Anne McLaren



Executive Summary

With the rapid development of new reproductive technologies, the possible kinds of research into early stages of human development dramatically changed. For present purposes, such research has been classified into two main categories, preimplantation research and postimplantation research, in recognition of developmental changes to the zygote after implantation.

An introductory section provides a developmental frame of reference for understanding not only the nature of the research, but also the potential legal and social implications. Differences from the pre-embryonic stage to the embryonic stage and between the embryo and fetus are explained. A chart provides details of development that occurs throughout the nine months of pregnancy.

Discussion includes an overview of postimplantation embryo research (therapeutic or clinical versus non-therapeutic research, chromosomal studies, normal and abnormal development, biochemical and chemical studies, and tissue transplantation) and of preimplantation embryo research (particularly concerning fertility and diagnosis of genetic and chromosomal defects).

Concerns about regulation are raised, with comparisons of guidelines developed in the Western world. U.K. guidelines and laws are discussed in detail, because embryo research is monitored more closely in the United Kingdom than elsewhere.

Addressing an implicit concern, the author concludes that pre-embryonic research during the next 5 to 10 years is likely to be a continuation and development of current lines of study (as described in this report).

Introduction

The Royal Commission on New Reproductive Technologies commissioned this paper to review the current status of human embryo research, taking into account the manner in which it has developed, its themes and its objectives, the laws or guidelines that regulate its conduct in different countries, and the possible directions in which it might develop in the future.

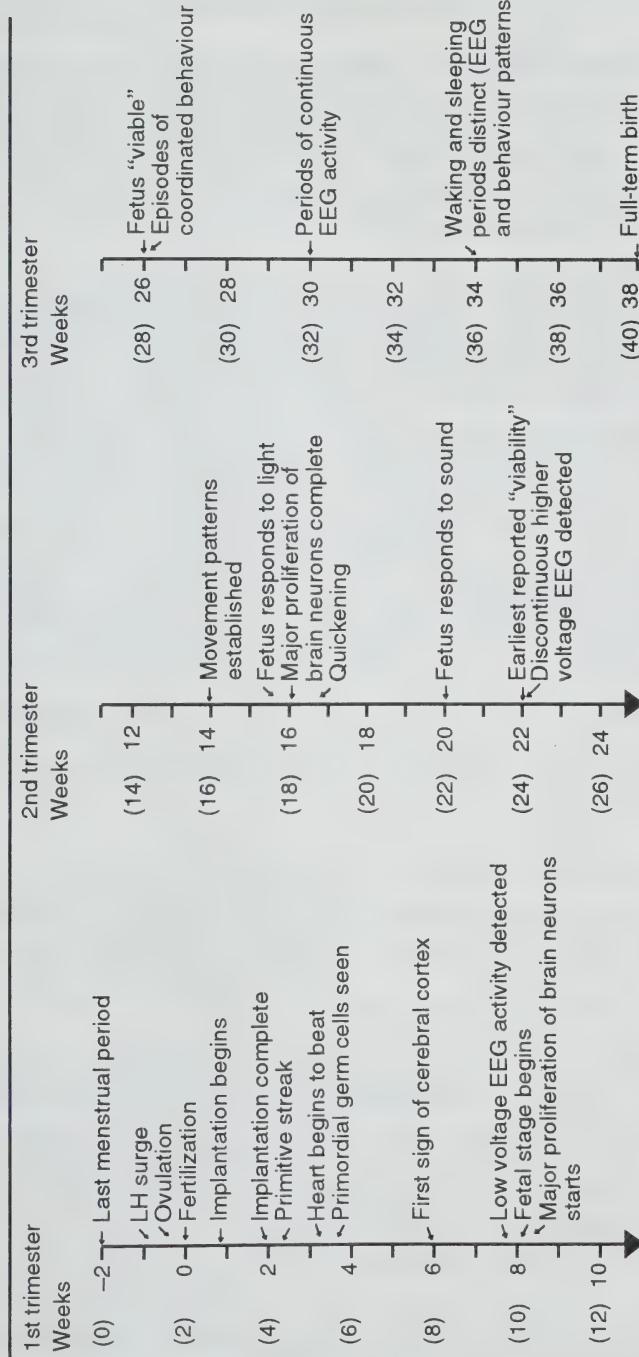
The information in the paper is drawn in particular from the situation in Western Europe, especially the United Kingdom. In part this is because of readier access to British sources of information than to those of other countries, but also because human embryo research is more closely monitored in Britain than elsewhere and more information is available.

Timing of Human Development

Fertilization, when the male and female gametes (sperm and egg) join together, can take place either inside the woman's body (*in vivo*) or in the laboratory (*in vitro*). The average interval between fertilization and the subsequent birth of the baby is 38 weeks. Unless otherwise stated, timings in this paper will be given in days or weeks from the time that the egg is fertilized, presumed in the absence of more precise information to be two weeks after the beginning of the woman's last menstrual period (LMP). In clinical reports, timings are often given in "weeks post-LMP." Figure 1 gives the timing of various relevant stages and events in human prenatal development. Three main stages of prenatal development are recognized: pre-embryonic, embryonic, and fetal (Moore 1988).

Pre-Embryonic Stage

The totality of cells and tissues derived from the fertilized egg, up to the 15-day stage when the embryo itself first forms as a distinct entity, is referred to in this paper as the zygote. Up to the 8- or 16-cell stage (three days post-fertilization), all of the cells of the zygote are believed to be equivalent to one another, and all are totipotent, in the sense that they are potentially able to contribute to any part of the future embryo or extra-embryonic membranes. During the next 10 days, the zygote grows to comprise thousands of cells, groups of which initiate the formation of

Figure 1. Chronology of Human Prenatal Development

Note: Times are given in weeks after fertilization, with weeks after last menstrual period (post-LMP) in parentheses.

Source: Based on A. McLaren, "Where to Draw the Line?" *Proceedings of the Royal Institution of Great Britain* 56 (1984), Figure 8.

various extra-embryonic structures. These assist the zygote in burrowing into the wall of the uterus in the process of implantation, which lasts from 7 to 14 days after fertilization.

The extra-embryonic membranes, which include the placenta (after-birth), amnion, yolk sac, and umbilical cord, are distinct from the embryo or fetus during the later stages of development and will not be discussed further in this paper. During the pre-embryonic stage of development, the cells that will give rise to extra-embryonic structures form part of the zygote and therefore will be considered below.

Embryonic Stage

The embryonic stage begins at 15 days after fertilization in a normal human pregnancy, when the primitive streak (a groove through which cells migrate to set up the primordium of the embryo) first appears on the embryonic plate, and it is arbitrarily regarded as ending at eight weeks after fertilization, after which the term "fetus" is used. The distinction between the embryonic and the pre-embryonic stage is not arbitrary: from the 15-day stage onwards, the embryo is isomorphic with the fetus, the baby, and the adult, so that it is possible to define which cells and tissues will contribute to the fetus and thence to the baby and which will contribute to the extra-embryonic membranes. This distinction is not possible before 15 days. The 15-day stage is also the point at which *individual* human development begins, because it is the last point at which monozygotic (so-called "identical") twinning can occur (Bulmer 1970). If two primitive streaks form on the embryonic plate rather than just one, two embryos will develop and, if both survive, twins will in due course be born. The embryonic period is characterized by active organogenesis and morphogenesis.

Fetal Stage

In a normal human pregnancy, the fetal stage is usually regarded as starting eight weeks after fertilization and ending at birth. By eight weeks the rudiments of all the organ systems have been laid down, so that the fetal stage is characterized mainly by maturation and growth. The eight-week fetus has the general appearance of a baby animal, with four limbs and a head, but it is still only about 3 cm in length.

The distinction between fetus and embryo is hallowed by antiquity and is widely used by clinicians, though it may have no scientific basis, and the eight-week dividing line seems arbitrary. Some authorities specify 10 weeks, in which case the distinction between embryo and fetus corresponds approximately to the distinction between the first and second trimester of the pregnancy.

Terminology

The number of terms that have been employed for the products of the fertilized egg during each of the above stages has led to some confusion.

From the scientific point of view, it is important that terms should be defined. Which terms are used is less important than that they should be used unambiguously.

The totality of cells and tissues derived from the fertilized egg up to the primitive streak stage when the embryo first forms as a distinct entity has been referred to not only as the zygote, but also as the pre-embryo, pro-embryo, ovum, or conceptus. The term "preimplantation embryo" is sometimes used in this sense, but it has also sometimes been used to describe the zygote up to the time when it begins to implant in the uterus (six to seven days after fertilization).

The U.K. committee that reported (United Kingdom, Advisory Group 1972) on the use of fetuses and fetal material for research (the Peel Report) applied the term "fetus" to every developmental stage from the fertilized egg through to birth. This usage is not helpful in the present context, and has not been adopted by the Commission.

The Warnock Committee, reporting in the United Kingdom in 1984 on *in vitro* fertilization (IVF) and human embryology, applied the term "embryo" to every developmental stage from the fertilized egg through to the fetal stage (United Kingdom, Committee of Inquiry 1984).

In this paper, where use of the term "embryo" during the pre-implantation period could lead to ambiguity, the term "zygote" will be used instead. From the primitive streak stage onwards, the terms "embryo" or "postimplantation embryo" will be used.

Research Approaches

Research on postimplantation embryos is very different from research during the pre-embryonic period, with respect to its objectives and manner of conduct, the regulations that govern it, and in particular the source of the embryonic material. Therefore, these two categories of human embryo research will be considered separately.

Research relating to the human fetus is reviewed elsewhere in the Commission's publications and, therefore, will be mentioned only in passing in the present paper.

Postimplantation Embryo Research

Source of Embryonic Material

Embryonic material may be obtained from spontaneous abortions, from induced abortions, or from ectopic pregnancies (in which implantation takes place outside the uterus). Embryos are occasionally found at hysterectomy or at autopsy.

Spontaneous Abortions

A pregnancy may terminate spontaneously because the embryo has died. Even if it can be recovered, it is likely to be degenerating, because expulsion from the uterus often takes place several days or even weeks after death. If, on the other hand, the pregnancy terminates because the uterus undergoes premature contractions, a living though non-viable embryo may be delivered. If the aborted material (mostly extra-embryonic membranes and blood) is collected, the embryo can occasionally be identified and examined, despite its very small size.

Induced Abortions

Induced termination of pregnancy is undertaken if the life or health (either physical or mental) of the mother is threatened, if the embryo is known to be or very likely to be suffering from some serious genetic or chromosomal defect or congenital malformation, or (in some countries) for social reasons. In 1981, of the 1.3 million induced abortions performed in the United States, 78 percent were between 6 and 11 weeks post-LMP (Fine 1988) (i.e., in the first trimester and mostly in the embryonic rather than the fetal stage).

In the first trimester, termination of pregnancy has traditionally been carried out by dilatation of the cervix and curettage to remove the uterine contents (D&C). Today, vacuum aspiration is widely used: for example, 94 percent of the abortions induced between 6 and 11 weeks in the United States in 1981 were done by vacuum aspiration (Fine 1988). With a D&C, smaller embryos are occasionally recovered more or less intact; with vacuum aspiration, all are reduced to fragments. Individual tissues can nonetheless often be distinguished and are usually in good condition so that they grow well in culture.

The United Kingdom's Medical Research Council Tissue Bank estimates that, at 10 weeks post-LMP (end of the embryonic period), tissue from the spinal column can be recovered in 85 percent of vacuum aspiration specimens, brain and eye tissue in 15 percent, and heart and liver tissue in 5 percent (Wong 1988). Such tissues as adrenal, kidney, lung, spleen, thymus, and stomach cannot be recovered at 10 weeks.

Hormonal methods are also used for inducing abortion. These involve either prostaglandin alone, or prostaglandin in combination with an anti-progestin such as RU-486. These methods result in maceration of the embryo and yield tissues in poorer condition that may fail to grow in culture (Wong 1988).

Ectopic Pregnancies

Ectopic pregnancies are terminated surgically to safeguard the life of the mother. They can provide a source of embryonic material in those cases in which diagnosis and intervention occur in the first trimester. Development of the embryo proceeds normally, at least in the early stages.

Obtaining Fetal and Embryonic Material

Countries such as Britain and the United States, in which termination of pregnancy is legal, and where extensive use is made of fetal and embryonic material for diagnostic and research purposes, have set up agencies mandated to organize the collection and distribution of this material in accordance with strict guidelines.

In Britain, the Medical Research Council set up its Tissue Bank in London in 1957, for the obtaining and supplying of human embryonic and fetal tissues for research (Wong 1988). It has been in continuous operation ever since. Between 1981 and 1986, there were 124 users, of whom 27 percent were virologists, 23 percent molecular biologists or geneticists, 17 percent immunologists, and 15 percent cell biologists.

The British Tissue Bank is financed entirely by the government: the obstetricians who supply the material receive no remuneration, and the research workers who receive it pay nothing but transport costs. All projects must be approved by an ethical committee before any material is supplied.

At least two non-profit procurement agencies existed in the United States in 1990. The National Disease Research Interchange, set up in 1980, has provided some 36 000 human tissue samples of all types to approximately 400 research workers studying some 80 different diseases (Ducat 1988). Between 1984 and 1987, 2 000 to 3 000 fetal and embryonic samples per year in total were supplied to some 23 research workers. Institutions (e.g., hospitals) are reimbursed for the use of their facilities to collect specimens; research workers are charged fees to cover costs, and they must have their projects approved by a board of scientists. The International Institute for the Advancement of Medicine, set up in 1986, operates in a very similar way (Bardsley 1988). Both of the above agencies follow strict ethical guidelines.

Status of Embryo (Postimplantation to Eight Weeks)

The human embryo is clearly not viable, in the sense of having reached a stage of maturity such that it is capable of continued independent existence. From the ethical point of view, it may nonetheless be relevant to inquire whether a particular embryo (either *in utero* or *in vitro*) is alive or dead.

At the level of the whole organism, the two most commonly used criteria of death are brain death (based on changes in the electrical activity of the brain) and heart death (based on irreversible cardiac arrest). At a lower level, tissues and cells may survive after the death of the organism. Once a tissue has ceased to function in an integrated manner, and communication between its constituent cells has broken down, it can no longer be regarded as surviving as a tissue. Its constituent cells may, however, still be alive, and may be capable of indefinite survival given appropriate culture conditions.

For the embryo (that is, the stage after implantation to eight weeks), brain death cannot be used as a criterion, because of the immaturity of the developing nervous system and its function. Some authorities define embryonic death as irreversible loss of function of the organism as a whole. Cardiovascular criteria may also be used, once the heart has started to beat and a blood circulation has become apparent (three to four weeks after fertilization). Before this stage, neither brain death nor heart death is applicable. Therefore, the embryo has to be regarded as a tissue, so that it may be considered to be alive as long as it continues to function in an integrated manner, and communication between its constituent cells is maintained.

Categories of Research

Anatomical Description

Historically, some of the first studies of human development during the embryonic stage were carried out on specimens recovered intact from women undergoing either curettage (e.g., Wilson 1945) or hysterectomy (e.g., Hertig and Rock 1944; Hertig et al. 1956). The hysterectomy studies, which yielded the best preserved specimens, are unlikely to be repeated or extended because the women were requested to have intercourse on particular days prior to the hysterectomy operation and such a procedure would not be regarded as ethical today. In any case, some have raised doubts as to the normality of these embryos because the women must have been under considerable stress and this could have affected their hormone levels.

Abnormal Embryonic Development

When material is recovered from spontaneous abortions, both the embryo and the extra-embryonic tissue (chorionic villi) may be subjected to morphological analysis in an attempt to diagnose the cause of the pregnancy failure.

Chromosomal Studies

Material from spontaneous abortions at this stage has also been extensively subjected to chromosomal analysis, and abnormal morphological development has been correlated with chromosome abnormalities. More than 50 percent of all first-trimester spontaneous abortions have been shown to have an abnormal karyotype; for some abnormalities, embryonic development is so poor that chromosomal analysis can be carried out only on chorionic villi. Comparisons between the chromosomal constitution of embryonic and extra-embryonic tissue can be informative: usually they are concordant, but cases of mosaicism have been reported.

To provide baseline data on the overall proportion of embryos that are chromosomally abnormal, chromosomal studies have been carried out on material from early postimplantation pregnancies terminated for social reasons (Yamamoto and Watanabe 1979; Burgoyne et al. 1991). (The

incidence of chromosomally abnormal embryos four to five weeks post-fertilization was about 5 percent.) Chromosomal studies have also been conducted on embryos from abortions induced after diagnosis of genetic abnormality by early chorionic villus sampling (CVS).

Biochemical and Molecular Studies

Embryonic material from induced abortions may be subjected to biochemical analysis (e.g., to study enzyme activity in normal embryos and those with specific genetic defects). Molecular analysis, for example the construction of complementary deoxyribonucleic acid (cDNA) libraries, can reveal which genes are expressed in which tissues at which stage of development, and it can aid in the isolation and characterization of gene defects responsible for human genetic diseases.

Because it is the most readily identifiable tissue in 7- to 10-week post-LMP embryos, much of the research use of embryonic material centres around neural tissue. Typical projects include biochemical studies of the early development of the human embryonic brain, preparation of a cDNA library from embryonic neural tissue, and cloning of a neuronal surface protein involved in neural migration. Comparisons are being carried out between gene expression in neural tissue from normal and Down syndrome embryos.

Other projects are concerned with embryonic heart development and actin and myosin gene expression in embryonic muscle. Aspects of early haemopoiesis, such as the appearance of haemopoietic stem cell growth factors, can be examined in embryonic liver.

Tissue Transplantation

Transplantation of human brain tissue (usually at 7 to 12 weeks post-LMP) into rats and monkeys suffering from a drug-induced condition similar to Parkinson's disease produced a dramatic improvement in motor coordination (Redmond et al. 1988; Brundin et al. 1988). The brain-stem area that contains the vital dopamine neuroblasts of the substantia nigra region could be identified in about 50 percent of early vacuum aspiration specimens. Using immunodeficient hosts, the survival, growth, and development of the dopamine neuroblasts have been followed, and the conditions for dopamine release optimized (Stromberg et al. 1986).

Research workers are also studying the possibility of using transplanted neural tissue to facilitate nerve regeneration. Animal experiments are already in progress using models of Alzheimer's disease, Huntington's disease, spinal cord injury, and neuroendocrine deficiencies: if the results look promising, the next step would be to graft human embryonic neural tissue into immunocompromised animal subjects.

Clinical (Therapeutic) Research

Therapeutic research is defined by some as the application of a new and untried procedure with the intention not only of advancing biomedical knowledge but also of curing or alleviating the condition of a particular

individual (as opposed to non-therapeutic research, which is aimed at advancing biomedical knowledge rather than treating any particular individual).

The above tissue transplantation studies on animal models of human diseases are, of course, aimed at developing new therapeutic approaches for clinical use. If the animal studies show promise, there is a strong incentive to move on to clinical trials. Until a new therapy has been validated and shown to be effective and free of damaging side-effects, all clinical use should be categorized as research, and the clinical trial designed as such.

The only embryonic tissues that are sufficiently developed to be likely to be used for therapeutic purposes are liver and neural tissue. The liver cells used to date for transplantation into heavily irradiated patients (those exposed at Chernobyl for example) have been fetal in origin, while those grafted into severe combined immunodeficiency (SCID) patients between 1971 and 1981 (O'Reilly et al. 1988) were from abortions carried out 8 to 14 weeks post-LMP (i.e., mostly fetal, but including some embryonic material). But SCID patients now are treated more often with bone marrow grafts from adult donors rather than with fetal or embryonic liver transplants.

For neural transplantation, first-trimester tissue (late embryonic and early fetal period) survives better than second-trimester tissue, and it contains cells at a stage of differentiation that is more appropriate for the therapeutic goals.

Clinical research projects involving the transplantation of immature human neural tissue into the brains of patients suffering from Parkinson's disease have been reported from Mexico (Madrazo et al. 1987), Cuba, Sweden (Lindvall et al. 1988), Britain (Hitchcock et al. 1988), and the United States. If a beneficial effect of treatment can be established, it will remain to be shown whether the transplanted cells are surviving, growing, and becoming functionally integrated into the host brain, whether they are merely providing a transient input of dopamine, or whether the grafting operation itself is exerting a placebo effect to bring about a temporary increase in motor function (Sladek and Shoulson 1988).

For the future, transplantation of immature neural tissue has been suggested as a possible therapeutic approach to other degenerative disorders of the human nervous system, such as Alzheimer's disease and Huntington's disease.

Aims and Future Directions

From the above outline of current research projects on human postimplantation embryonic material, it is evident that the main aims of the research are twofold:

- to elucidate the molecular basis of early human development, with the aim of understanding the etiology of abnormal development; and
- to explore the possibilities of using embryonic tissue, in particular neural tissue, to repair defects that arise later in development, particularly defects affecting the nervous system.

Because only a very small amount of progress has been made toward achieving either goal so far, it seems likely that research will continue to target these two objectives for several decades to come.

On the other hand, the likelihood of postimplantation embryos themselves being the object of therapeutic research seems small. If the current efforts at somatic gene therapy are successful for some genetic diseases, there may eventually be attempts to treat early-acting diseases before rather than after birth. Such attempts would surely focus on the fetal rather than the embryonic stage of development. If any attempts at genetic manipulation for therapeutic purposes were to be made on postimplantation embryos, careful consideration would have to be given to distinguishing between somatic and germ line gene therapy. By analogy with the mouse, it is likely that the germ cell lineage does not become established until some time early in the embryonic period, after the primitive streak has formed. An intervention at such an early stage that was intended to treat somatic cells might therefore modify genes in germ cells also.

Ethical Considerations

The ethical issues raised by human postimplantation embryo research are no different from those raised by research on human preivable fetal material. Although the products of an abortion are not regarded as being legally the property of the mother, it is usual to obtain her consent for their use for research. To avoid any possibility that a woman might deliberately terminate a pregnancy to provide fetal or embryonic material, for example for tissue transplantation, it is an ethical requirement that the decision to have an abortion should precede and be independent of that consent and that no directed designation of recipients for such tissue be permitted. It may also be desirable that the persons carrying out the research should be separate from the doctor responsible for the abortion, though this is more important for tissue transplantation than for other types of research (e.g., chromosome studies). It is not ethically acceptable for the products of an abortion to be sold.

Regulation

The use of fetal and postimplantation embryonic tissue for research is governed in the United Kingdom by the recommendations of the government committee chaired by Professor Polkinghorne (United Kingdom,

Committee to Review 1989). The committee recommended that guidance on the use of such tissue should be based on ethical principles embodied in a code of practice (Code of Practice on the Use of Fetuses and Fetal Material in Research and Treatment) rather than in legislation (*ibid.*, 22-24). The code specifies that women from whom fetal or embryonic tissue is obtained should give their explicit consent to its being used for research. They should not be informed of the specific use that may be made of the tissue, nor whether it is to be used at all (this recommendation is the subject of some debate). It follows that women cannot designate a recipient of the tissue. The consent should not be sought until after the woman has decided to have an abortion. The doctors who supply the tissue should be separate from the doctors and scientists who carry out the research. There should be no monetary exchange for the tissue, and all research projects must first be approved by an ethical committee.

Most other European countries also insist that relevant research projects should be approved by an ethical committee. In Spain, a law passed in 1988 specifies that research on dead human embryos or fetuses older than 14 days will be allowed only if the project has been authorized by the public authorities or by a national commission set up for that purpose.

In its December 1988 report to the National Institutes of Health, the U.S. Human Fetal Tissue Transplantation Research Panel, set up to consider the ethical and legal problems raised by human fetal tissue transplantation research, recommended the following guidelines for any use of human fetal or embryonic tissue for transplant research and therapy:

1. The decision to terminate a pregnancy and the procedures of abortion, including the certification of death, should be independent from the retrieval and use of fetal or embryonic tissue and should not involve the same personnel.
2. Payments and other forms of remuneration and compensation associated with the procurement of fetal or embryonic tissue should be prohibited, except payment for reasonable expenses occasioned by the actual retrieval, storage, preparation, and transportation of the tissues.
3. Potential recipients of such tissues, as well as research and health care participants, should be properly informed as to the source of the tissues in question.
4. Fetal or embryonic tissues should not be used without the prior consent of the pregnant woman, and should not be used if the father objects.
5. Procedures must be adopted that accord human fetal and embryonic tissue the same respect accorded other cadaveric human tissues entitled to respect. (U.S. National Institutes of Health 1988, vol. 1, 1)

The panel recommended to the National Institutes of Health that the support of transplantation of human fetal (including postimplantation embryonic) tissue for purposes of research on therapy was acceptable public policy. However, the recommendation was not adopted, and a moratorium on federal funding of such research was continued until January 1993, when it was reversed by the Clinton administration.

Preimplantation Embryo Research

Source of Embryonic Material

Fertilized eggs and early cleavage stages could in principle be obtained by washing out a woman's fallopian tubes during the first three days after coitus. Later cleavage stages and the hollow "blastocyst" stages are located in the uterus during the following three days, but they have not yet begun to implant. They too could be recovered by washing out the uterus through the cervix, a relatively simple procedure that has been used to obtain donor embryos for transfer (Buster et al. 1985) but which has some drawbacks. To date it has played no part in human embryo research.

All cleavage-stage human embryos used in research today are derived from IVF. Unfertilized eggs (oocytes) are recovered, either by laparoscopy or under ultrasound, just before they are due to be shed from the woman's ovaries. They can be fertilized outside the body, and will develop for a few days *in vitro* just as they would *in vivo*, though somewhat more slowly. Usually the woman has been treated with hormones to increase the number of eggs available.

For therapeutic purposes, all the eggs recovered are exposed to sperm, since not all will be successfully fertilized and not all of those fertilized will undergo cleavage. A maximum of three or four zygotes will be replaced in the woman's uterus, usually at the four- to eight-cell stage. Replacement of larger numbers does not increase the chances of pregnancy proportionately, and runs an unacceptably high risk of producing "multiple pregnancies" (i.e., twins, triplets, and higher multiples). These endanger the woman and the maintenance of her pregnancy, as well as decreasing the chances of survival and normal development of any babies that are born. Any zygotes in excess of the number replaced are termed "spare" or "surplus" zygotes. Now that cryopreservation is possible, the couple often keeps the "surplus" zygotes for their own attempts to have a child in future cycles, but they may be available, with the couple's consent, for other purposes.

An alternative source of zygotes used in research involves unfertilized eggs donated by women undergoing gamete intrafallopian transfer (GIFT) or other relevant operative procedures (e.g., sterilization by tubal ligation). These can be fertilized *in vitro* and used for the study of fertilization itself or the early stages of development, before "spare" zygotes become available.

As more couples choose the option of cryopreserving their "spare" zygotes for their own future use, donated oocytes fertilized *in vitro* may become an increasingly important source of pre-embryonic material.

For some types of research (e.g., microinjection of sperm into the egg), eggs that have failed to fertilize during a therapeutic IVF procedure could be used. Eggs that have been fertilized by more than one sperm (polyspermic) and that would therefore develop abnormally if replaced in the uterus are also used for research, but this involves the assumption that their development before implantation would be unaffected.

Some success has been recorded in achieving *in vitro* maturation and fertilization of immature oocytes recovered from infertile women, as part of their treatment. Large numbers of immature oocytes could be obtained from fetal ovaries after abortion, or from the ovaries of women who have died, but so far no attempts have been made to mature and fertilize these for research purposes. Even if it were to be judged ethically acceptable on other grounds, donor consent would also pose a problem.

Status of Embryo (At Preimplantation Stage)

The zygote, at least from the 8- to 16-cell stage onwards, may be regarded as a tissue, so that it may be considered to be alive as long as it continues to function in an integrated manner and communication between its constituent cells is maintained. As with a tissue, the constituent cells may survive after the death of the zygote. Up to the 8- to 16-cell stage, no criterion other than that of cell death can be applied. A cell is said to be dead when its outer membrane has lost its integrity, so that the cell can no longer function as an independent entity.

From an ethical and regulatory point of view, no distinction has been made between research on live versus dead preimplantation embryos. The term "non-viable" is sometimes applied to polyspermic embryos, but this usage is misleading. Polyspermic embryos would continue to develop if replaced in a uterus, though few would survive to term. Neither polyspermic nor normal monospermic embryos would be capable of long-term development unless replaced in a uterus.

History and Ethical Considerations

During the 1940s, 1950s, and 1960s, a few sporadic reports appeared in which a cleavage-stage human embryo had developed following the exposure of an oocyte to sperm (e.g., Menkin and Rock 1948; Shettles 1955). No convincing evidence was presented that cleavage was due to fertilization rather than to parthenogenesis. At the same time, studies were being carried out all over the world on IVF and embryo transfer in various animal species (e.g., rabbit, mouse, cattle, sheep). Embryo transfer (from embryo flushing, not from IVF) became a routine procedure in laboratory and farm animals and formed the basis of much experimentation that enhanced our understanding of early mammalian development and

embryo-maternal interactions. Animals born following IVF and embryo transfer showed no abnormalities and were fully fertile.

The first systematic research on human IVF was carried out from the late 1960s onwards by Edwards and Steptoe in England. Using Steptoe's laparoscopic expertise to recover pre-ovulatory oocytes from the ovaries of infertile women and Edwards' experience with embryo culture in mice and rabbits, they worked for years to establish the appropriate conditions for human IVF, developing an appropriate medium for the culture of human embryos and the appropriate uterine environment for successful embryo transfer and subsequent pregnancy (Edwards 1980). The hundreds of eggs used were donated for research by women with tubal problems. The birth in 1978 of Louise Brown, the world's first IVF baby, was followed by efforts to set up IVF clinics in many parts of the world. By now, 15 years later, tens of thousands of such babies have been born. IVF is now used widely as a clinical procedure for the treatment of male as well as female infertility.

From the beginning, Edwards realized the ethical and social implications of his work (Edwards and Sharpe 1971; Edwards 1974), and he made many attempts to get them debated publicly. However, it was not until 1982 that the British government set up a committee, chaired by Mary Warnock, to enquire into the question of human fertilization and embryology, with particular reference to the impact of new biomedical advances, and to make recommendations on possible legislation. Many individuals and organizations submitted their views to the committee, and debate continued after the publication of the Warnock Report (United Kingdom, Committee of Inquiry 1984). Similar committees of inquiry have since been set up in other countries, and debate has become worldwide.

One of the most controversial issues has been the ethical status of human IVF embryo research. Two fundamentally different ethical standpoints have become crystallized, with rather few intermediate positions.

One view regards the fertilized human egg as a human person whose integrity and life must be given full protection. The only research that would be ethically acceptable would be therapeutic research undertaken for the benefit of that particular embryo. Some (but not all) of those who hold this view regard IVF as an acceptable treatment for infertility: they recognize that non-therapeutic embryo research was carried out in order to develop the technology, but they would not license any further such research.

The contrasting view, which was the majority view of the Warnock committee, regards the fertilized egg, though indubitably human and hence deserving of respect, as not yet a human person to be accorded the same rights and protections that would be given to a newborn child. Therefore, certain categories of non-therapeutic embryo research, under strict regulation, would be ethically acceptable provided that the embryo was not destined to be replaced in a uterus and had no possibility of further development. This view would regard rights and protections as being acquired progressively as embryonic development advances. The Warnock

committee selected 14 days after fertilization as an appropriate limit beyond which no human zygote should be maintained *in vitro*. This corresponds to a stage just prior to gastrulation, the end of the pre-embryonic stage: a stage when the postimplantation embryo that will develop into the fetus can first be recognized as an entity separate from the extra-embryonic membranes. It also corresponds to the last stage at which monozygotic twinning can occur (i.e., the stage at which individuality is established).

The first view allows therapeutic but not non-therapeutic research; for the second view the reverse is true. An often-quoted hypothetical example relates to the egg that has been fertilized by two sperm so it has an extra male nucleus. Adherents of the first view would allow experimental attempts to remove such extra nuclei by micromanipulation provided that the intention was to replace the resulting embryo in the uterus. Adherents of the second view would allow the same experimental manipulations as part of an approved research project, but only if the resulting embryo was *not* replaced in the uterus. Clinical use of the procedure would be disallowed until sufficient evidence that it did not damage the embryo had accumulated.

Regulation

In Britain, the Warnock report recommended in 1984 that legislation should be introduced to monitor and control research involving human IVF. In 1985, the Voluntary (later termed Interim) Licensing Authority (VLA) was set up by the Medical Research Council and the Royal College of Obstetricians and Gynaecologists to administer a system of voluntary regulation of IVF treatment and research, according to a set of guidelines closely modelled on the recommendations of the Warnock report. The guidelines specify that no research project should be started before being approved by the VLA; the research should be clinically relevant and scientifically valid; the consent of the donors must always be obtained, as well as the consent of the local ethical committee; certain lines of work including any modification of the genetic constitution were prohibited; no fertilized egg should be grown for more than 14 days *in vitro*; and no fertilized egg that was the subject of experimentation should be replaced in the uterus.

Five years later, legislation was passed by the U.K. Parliament that included provisions to regulate human IVF research. Members of Parliament were given a free vote as to whether licensed research should be permitted. In the Upper House, the vote in favour of allowing research was 234 to 80; in the House of Commons it was 364 to 193. An amendment was proposed to prohibit the fertilization of eggs for research purposes (sometimes termed "special creation of embryos for research"). This was defeated by 214 votes to 80 in the Upper House, and by 246 votes to 208 in the Commons. The Human Fertilisation and Embryology Bill was finally approved by the House of Commons in June 1990, by 303 votes to

65. The provisions regulating IVF research under the act are similar to those drawn up by the VLA; they are now enforced by a statutory licensing authority, the Human Fertilisation and Embryology Authority (HFEA). It is now a criminal offence to undertake IVF research without a licence, or to contravene the act in any way (e.g., by culturing a human embryo for more than 14 days after fertilization).

In other European countries, the laws or guidelines that have been adopted depend on which of the two ethical views outlined above is taken by the authorities. For example, Spain and Portugal have passed legislation very similar to that of the United Kingdom, except that in Portugal the fertilization of eggs donated for research purposes is prohibited. Belgium and the Netherlands do not yet have legislation, but their guidelines for IVF research are similar to those in the Warnock report; only Belgium allows donated eggs to be fertilized for research purposes.

In France, the National Consultative Ethics Committee has considered IVF research and has concluded that no type of research should be prohibited, though all projects must be submitted to the committee for approval. Furthermore, the permitted duration of culture could vary from one project to another; no embryo should be produced exclusively for research; and no embryo used for research should be replaced in the uterus. At present, there is a moratorium on research aimed at preimplantation genetic diagnosis (see next section).

Countries in which the contrasting view prevails include Germany, Ireland, and Norway. In all of these, legislation exists that prohibits any non-therapeutic human IVF research.

The Ethics Committee of the American Fertility Society (1988) has issued a set of guidelines similar to those adopted by the VLA.

Categories of Research

Most research on IVF embryos is conducted in Britain, France, Australia, and the United States, though certain states of both Australia and the United States have laws prohibiting such research. In Britain, a list of all current research projects is published annually by the HFEA (formerly by the VLA). The projects, which are required to be clinically relevant, are directed toward the alleviation of infertility, the regulation of fertility, and the diagnosis of genetic and chromosomal defects. As they are representative of projects carried out in other countries also, the research will be reviewed under these three headings.

Certain areas of research (e.g., human-animal hybridization, cloning by nuclear substitution, genetic manipulation) are prohibited in Britain and in most other countries. Although therapeutic research is allowed in those countries that ban non-therapeutic research, no therapeutic IVF research projects in progress are known to this author.

Alleviation of Infertility

Most of the research projects in this category are concerned with increasing the success rate of IVF itself, which is still rather low. Studies have been carried out on different hormonal methods of stimulating egg development, different schedules of when to recover the eggs from the ovary, and how long to keep them before mixing them with sperm and in what sort of fluid, to ensure optimal egg maturation (e.g., Templeton et al. 1986; Edgar et al. 1987). Although all these projects are concerned with eggs that are unfertilized initially, they have to be fertilized *in vitro* so that the fertilization rate, cleavage rate, and chromosome constitution can be checked. Such projects have to be undertaken on unfertilized eggs donated specifically for research.

One discovery in particular has contributed to the improved pregnancy rate of IVF over the last few years, namely the importance of temperature (Pickering et al. 1990). When the unfertilized egg is cooled to room temperature, the spindle fibres holding the chromosomes depolymerize. In the mouse, this process is reversible, but the human egg is more sensitive: when the egg is warmed, the spindle often does not reform normally, so chromosomes may be lost after fertilization.

Other projects have been concerned with the cryopreservation of unfertilized eggs (Hunter et al. 1991; Pickering et al. 1991; Bernard et al. 1992). Following research on the techniques of embryo cryopreservation using both animal and human embryos, low-temperature storage of human embryos at various stages of preimplantation development has been carried out successfully for several years (Trounson and Mohr 1983; Zeilmaker et al. 1984; Cohen et al. 1985; Testart et al. 1986; Ashwood-Smith 1986), but the temperature sensitivity of the spindle holding the chromosomes of the egg before fertilization has meant that particular care has to be taken to devise a freezing and thawing protocol that is compatible with normal development after fertilization. Although a small number of pregnancies have been reported following transfer of embryos derived from eggs frozen prior to fertilization (Chen 1986; van Uem et al. 1987), the HFEA does not yet permit the procedure to be used clinically. If in the future it is judged to be safe for clinical use, it will aid the logistics of IVF. It will also enable, for example, young women undergoing cancer therapy to store their eggs for future use.

Many early miscarriages are associated with chromosomal abnormalities of the embryo. The possibility of correlating these abnormalities with some aspect of egg maturation or fertilization lends great importance to the many karyotyping studies that have been carried out on IVF embryos (Angell et al. 1983, 1988; Michelmann and Mettler 1985; Plachot et al. 1988; Wimmers and Van der Merwe 1988). Studies of the incidence of various types of embryonic abnormality have also been carried out (Winston et al. 1991).

Preliminary attempts have been made to remove a male pronucleus microsurgically from a tripronucleate human fertilized egg (Rawlins et al.

1988). Some development has been observed following this procedure (Malter and Cohen 1989b), but until the male pronuclei can be identified with certainty, and the risks of aneuploidy, molar pregnancy, and other forms of abnormal development have been evaluated, it is unlikely to be used clinically.

Studies on the metabolism of IVF embryos after fertilization (Leese et al. 1986; Wales et al. 1987; Hardy et al. 1989b) are of particular value, not only for the light they throw on designing improved culture media containing appropriate nutrients, but also for the possibility they offer of monitoring the normality of cleavage-stage embryos in a non-invasive manner. Secretion of chorionic gonadotropin (hCG) could be another indication of normal development. Though messenger ribonucleic acid (mRNA) for hCG has been shown to be present at the eight-cell stage (Bonduelle et al. 1988), the hormone itself was not detected in the culture medium until several days later (Fishel et al. 1984), which is too late to be a useful indication of development potential after transfer to the uterus. The effect of introducing growth factors to the culture medium is also being examined.

Nearly half of all infertility problems can be attributed to poor sperm quality in the male partner. IVF has been used successfully to treat such conditions, especially when the sperm count is very low. Much IVF research is concerned with assisting fertilization when sperm motility is poor, and the couple could otherwise only achieve a pregnancy using donor insemination. Thinning or cutting the zona pellucida that surrounds the egg has resulted in some pregnancies going to term (Depypere et al. 1988; Gordon et al. 1988; Cohen et al. 1989; Malter and Cohen 1989a, 1989c; Malter et al. 1989). When motility is lower still or absent, fertilization may be achieved by microinjecting sperm through the zona pellucida into the perivitelline space (Lassalle et al. 1987; Laws-King et al. 1987; Ng et al. 1988, 1989) or even into the cytoplasm of the egg itself (Sathananthan et al. 1989), but not enough studies have been done to show whether or not this procedure damages the egg and impairs its development.

In those parts of the world where embryo research is permitted, it is the task of the ethical committee or statutory authority to decide when sufficient research evidence of safety and efficacy has accumulated to justify allowing a novel procedure to be used clinically.

Regulation of Fertility

Gametes form an obvious target for contraception, but any approach aimed at blocking fertilization would need to be tested *in vitro*. A steroid method that interfered with spermatogenesis could only be considered for use if any remaining sperm proved incapable of achieving fertilization of donated eggs, rather than producing abnormal fertilization. One research project in progress at the present time is aimed at the development of a contraceptive vaccine able to block sperm binding to the zona pellucida (Henderson et al. 1987). Antibodies against the zona polypeptide have been

shown to prevent binding of sperm *in vitro*. In another study, the possible contraceptive effect of the antiprogestin RU-486 was examined (Messinis and Templeton 1988).

Diagnosis of Genetic and Chromosomal Defects

For couples at high risk of having a child with a severe genetic disease due to a single-gene defect (e.g., beta-thalassemia, Duchenne muscular dystrophy, cystic fibrosis, Lesch-Nyhan syndrome), studies are being carried out to assess the feasibility of preimplantation diagnosis. Removal of one or two cells at the 8- to 16-cell stage, or a few extra-embryonic cells at the blastocyst stage, would enable diagnosis to be carried out, revealing whether or not each biopsied embryo was affected by the genetic defect in question. Only unaffected embryos would then be replaced in the uterus.

For biopsy at the 8- to 16-cell stage, determinations of cell number and distribution of cells between inner cell mass and trophectoderm in the resulting blastocysts established that the procedure was unlikely to prejudice subsequent development (Handyside et al. 1989). Trophectoderm biopsy on human embryos at the blastocyst stage has been carried out by Dokras et al. (1990), but has not yet been used clinically. Baseline data on cell death and allocation between inner cell mass and trophectoderm have been collected for human blastocysts developed *in vitro* by Hardy et al. (1989a).

Although preimplantation diagnosis had been discussed for some years (Penketh and McLaren 1987), it did not become a practical possibility until the polymerase chain reaction (PCR) technique introduced by Saiki et al. (1988) had been developed to a level such that DNA from small numbers of cells or even single cells from cleavage-stage embryos could be amplified sufficiently to yield accurate and reliable diagnoses at the DNA level. Using this technique, DNA diagnostic research projects have been carried out on human embryo material for beta-thalassemia (Varawalla et al. 1991) and sickle-cell disease (Pickering et al. 1992). The feasibility of using PCR for preimplantation diagnosis of cystic fibrosis is also being explored (Coutelle et al. 1989). Since PCR is easier on repeated than on single-copy DNA, Handyside et al. (1990) used Y-chromosome repeated sequences to determine the sex of biopsied embryos from women who were carriers of X-linked genetic diseases such as Duchenne muscular dystrophy and fragile X syndrome. The male offspring were at 50 percent risk of being affected, whereas the females were not at risk of being affected.

An alternative diagnostic procedure involves *in situ* hybridization using labelled DNA probes (West 1989). West et al. (1987) were able to determine the sex of human eight-cell embryos by this means, using a radiolabelled probe for a repeated Y-linked sequence. A similar approach, but using non-radioactive *in situ* hybridization, was reported by Penketh et al. (1989). Recently, the sensitivity of the technique has been greatly increased, so that single-copy genes can now be identified, using fluorescent probes. Since both X- and Y-chromosome probes can be used on the same cell

(Griffin et al. 1992), this offers a much more reliable method of determining sex. Also, probes for autosomal genes would allow embryos carrying autosomal monosomies and trisomies (the most common chromosome abnormalities) to be identified.

Possible additional targets for genetic analysis would be the two polar bodies, products of the two successive meiotic divisions of the oocyte. For women who were carriers of a genetic disease, if a first polar body lacked the normal gene and contained only the defective gene, it would establish that the corresponding oocyte lacked even one copy of the defective gene, hence an embryo resulting from its fertilization would be unaffected. This approach has been attempted by Verlinsky and his colleagues (Strom et al. 1990; Verlinsky et al. 1990, 1992), but no pregnancies have resulted, and no research has been carried out to explore whether removal of a first polar body prejudices the subsequent development of the embryo. Chromosomal or *in situ* analysis of the second polar body could yield information on monosomies, and on trisomies such as Down syndrome.

For genes whose products are present in very early embryos, such as glucose phosphate isomerase (West et al. 1989), ultra-sensitive enzyme micro-assays can be used to detect a genetic defect in an embryo biopsy. This approach has been attempted for hypoxanthine phosphoribosyltransferase, the enzyme that is deficient in the rare but extremely serious Lesch-Nyhan syndrome. The results do not look promising (Braude et al. 1989), possibly because in the human embryo, unlike the mouse, the embryonic genome does not start to be expressed until the four- to eight-cell stage (Braude et al. 1988). Any enzyme present during cleavage may therefore be of maternal rather than embryonic origin. Monitoring of gene expression in normal as well as abnormal embryos (Artley et al. 1992) is important both in this context and in relation to improving the success rate of IVF.

Future Perspectives

If continued embryo research leads to a continued improvement in the success rate of IVF and if preimplantation diagnosis proves accurate and safe, more couples at risk of having a baby with a serious genetic disease may opt for preimplantation diagnosis rather than for CVS or amniocentesis, in order to avoid the possible termination of a pregnancy. More research projects are therefore likely to be carried out on biopsy methods, including polar body removal.

Sexing of embryos for social rather than genetic reasons is not regarded as ethically acceptable. Sexing using postimplantation prenatal diagnosis is nonetheless carried out for social reasons in many parts of the world, and pregnancies of the unwanted sex are terminated. Even in those countries where the male is more highly valued, this practice is unlikely to spread to preimplantation diagnosis, because within a few years the simpler

method of separating X- and Y-bearing sperm by flow-sorting is likely to become an option.

Although all sets of guidelines that have been drawn up so far regard genetic manipulation of IVF embryos as ethically unacceptable, there may in the future be suggestions of undertaking germ line gene therapy. This would, however, be very unlikely to be attempted if preimplantation genetic diagnosis is successful. Couples who are genetically at risk produce unaffected as well as affected embryos; replacing the unaffected embryos would probably be preferred to manipulating the affected embryos.

It has been suggested that eggs fertilized *in vitro* might be cultured up to an embryonic stage at which the cells were sufficiently differentiated to be transplanted for therapeutic purposes. At present, culture systems do not exist that would make this possible. It would also contravene the present guidelines that specify a limit of 14 days for the culture of eggs fertilized *in vitro*, and it is not clear what advantages using such tissue would have over using that recovered from early induced abortions.

Conclusion

In conclusion, the research projects that will be carried out on human embryos during the pre-embryonic period in the course of the next 5 to 10 years are likely to be a continuation and development of those that are in progress at the present time, as described in this report.

References

American Fertility Society. Ethics Committee. 1988. "Ethical Considerations of the New Reproductive Technologies." *Fertility and Sterility* 49 (Suppl. 1).

Angell, R.R., et al. 1983. "Chromosome Abnormalities in Human Embryos After *In Vitro* Fertilization." *Nature* 303: 336-38.

—. 1988. "Chromosome Anomalies in Early Human Embryos." *Journal of Reproduction and Fertility* (Suppl. 36): 73-81.

Artley, J.K., P.R. Braude, and M.H. Johnson. 1992. "Gene Activity and Cleavage Arrest in Human Pre-Embryos." *Human Reproduction* 7: 1014-21.

Ashwood-Smith, M.J. 1986. "Cryopreservation of Human Embryos." *Human Reproduction* 1: 319-32.

Bardsley, J.S., Jr. 1988. "Summary of Testimony: Procurement of Human Fetal Cadaver Tissue for Biomedical Research." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

Bernard, A., et al. 1992. "Fertilization and Embryonic Development of Human Oocytes After Cooling." *Human Reproduction* 7: 1447-50.

Bonduelle, M.-L., et al. 1988. "Chorionic Gonadotropin- β mRNA, a Trophoblast Marker, Is Expressed in Human 8-Cell Embryos Derived from Tripromonucleate Zygotes." *Human Reproduction* 3: 909-14.

Braude, P., V. Bolton, and S. Moore. 1988. "Human Gene Expression First Occurs Between the Four- and Eight-Cell Stages of Preimplantation Development." *Nature* 332: 459-61.

Braude, P.R., et al. 1989. "Measurement of HPRT Activity in the Human Unfertilized Oocyte and Pre-Embryo." *Prenatal Diagnosis* 9: 839-50.

Brundin, P., et al. 1988. "Human Fetal Dopamine Neurons Grafted in a Rat Model of Parkinson's Disease: Immunological Aspects, Spontaneous and Drug-Induced Behaviour, and Dopamine Release." *Experimental Brain Research* 70: 192-208.

Bulmer, M.G. 1970. *The Biology of Twinning in Man*. Oxford: Clarendon Press.

Burgoyne, P.S., K. Holland, and R. Stephens. 1991. "Incidence of Numerical Chromosome Anomalies in Human Pregnancy Estimation from Induced and Spontaneous Abortion Data." *Human Reproduction* 6: 555-65.

Buster, J.E., et al. 1985. "Biologic and Morphologic Development of Donated Human Ova Recovered by Nonsurgical Uterine Lavage." *American Journal of Obstetrics and Gynecology* 153: 211-17.

Chen, C. 1986. "Pregnancy After Human Oocyte Cryopreservation." *Lancet* (19 April): 884-86.

Cohen, J., et al. 1985. "Pregnancies Following the Frozen Storage of Expanding Human Blastocysts." *Journal of In Vitro Fertilization and Embryo Transfer* 2: 59-64.

—. 1989. "Partial Zona Dissection of Human Oocytes When Failure of Zona Pellucida Penetration Is Anticipated." *Human Reproduction* 4: 435-42.

Coutelle, C., et al. 1989. "Genetic Analysis of DNA from Single Human Oocytes: A Model for Preimplantation Diagnosis of Cystic Fibrosis." *British Medical Journal* (1 July): 22-24.

Depypere, H.T., et al. 1988. "Comparison of Zona Cutting and Zona Drilling as Techniques for Assisted Fertilization in the Mouse." *Journal of Reproduction and Fertility* 84: 205-11.

Dokras, A., et al. 1990. "Trophectoderm Biopsy in Human Blastocysts." *Human Reproduction* 5: 821-25.

Ducat, L. 1988. "The National Disease Research Interchange." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

Edgar, D.H., K.M. Whalley, and J.A. Mills. 1987. "Effects of High-Dose and Multiple-Dose Gonadotropin Stimulation on Mouse Oocyte Quality as Assessed by Preimplantation Development Following In Vitro Fertilization." *Journal of In Vitro Fertilization and Embryo Transfer* 4: 273-76.

Edwards, R.G. 1974. "Fertilization of Human Eggs In Vitro: Morals, Ethics and the Law." *Quarterly Review of Biology* 49: 3-26.

—. 1980. *Conception in the Human Female*. London: Academic Press.

Edwards, R.G., and D.J. Sharpe. 1971. "Social Values and Research in Human Embryology." *Nature* 231: 87-91.

Fine, A. 1988. "The Ethics of Fetal Tissue Transplants." *Hastings Center Report* 18 (June-July): 5-8.

Fishel, S.B., R.G. Edwards, and C. Evans. 1984. "Human Chorionic Gonadotropin Secreted by Preimplantation Embryos Cultured In Vitro." *Science* 223: 816-18.

Gordon, J.W., et al. 1988. "Fertilization of Human Oocytes by Sperm from Infertile Males After Zona Pellucida Drilling." *Fertility and Sterility* 50: 68-73.

Griffin, D.K., et al. 1992. "Dual Fluorescent In-Situ Hybridisation for Simultaneous Detection of X and Y Chromosome-Specific Probes for the Sexing of Human Preimplantation Embryonic Nuclei." *Human Genetics* 89: 18-22.

Handyside, A.H., et al. 1989. "Biopsy of Human Preimplantation Embryos and Sexing by DNA Amplification." *Lancet* (18 February): 347-49.

—. 1990. "Pregnancies from Biopsied Human Preimplantation Embryos Sexed by Y-Specific DNA Amplification." *Nature* 344: 768-70.

Hardy, K., A.H. Handyside, and R.M.L. Winston. 1989a. "The Human Blastocyst: Cell Number, Death and Allocation During Late Preimplantation Development In Vitro." *Development* 107: 597-604.

Hardy, K., et al. 1989b. "Non-Invasive Measurement of Glucose and Pyruvate Uptake by Individual Human Oocytes and Preimplantation Embryos." *Human Reproduction* 4: 188-91, 348.

Henderson, C.J., P. Braude, and R.J. Aitken. 1987. "Polyclonal Antibodies to a 32-kDa Deglycosylated Polypeptide from Porcine Zonae Pellucidae Will Prevent Human Gamete Interaction In Vitro." *Gamete Research* 18: 251-65.

Hertig, A.T., and J. Rock. 1944. "On the Development of the Early Human Ovum, with Special Reference to the Trophoblast of the Previllos Stage: A Description of 7 Normal and 5 Pathologic Ova." *American Journal of Obstetrics and Gynecology* 47: 149-84.

Hertig, A.T., J. Rock, and E.C. Adams. 1956. "A Description of 34 Human Ova Within the First 17 Days of Development." *American Journal of Anatomy* 98: 435-93.

Hitchcock, E.R., et al. 1988. "Embryos and Parkinson's Disease." *Lancet* (4 June): 1274.

Hunter, J.E., et al. 1991. "Fertilization and Development of the Human Oocyte Following Exposure to Cryoprotectants, Low Temperatures and Cryopreservation: A Comparison of Two Techniques." *Human Reproduction* 6: 1460-65.

Lassalle, B., A.M. Courtot, and J. Testart. 1987. "In Vitro Fertilization of Hamster and Human Oocytes by Microinjection of Human Sperm." *Gamete Research* 16: 69-78.

Laws-King, A., et al. 1987. "Fertilization of Human Oocytes by Microinjection of a Single Spermatozoon Under the Zona Pellucida." *Fertility and Sterility* 48: 637-42.

Leese, H.J., et al. 1986. "Uptake of Pyruvate by Early Human Embryos Determined by a Non-Invasive Technique." *Human Reproduction* 1: 181-82.

Lindvall, O., et al. 1988. "Fetal Dopamine-Rich Mesencephalic Grafts in Parkinson's Disease." *Lancet* (24 December): 1483-84.

McLaren, A. 1984. "Where to Draw the Line?" *Proceedings of the Royal Institution of Great Britain* 56: 101-21.

Madrazo, I., et al. 1987. "Open Microsurgical Autograft of Adrenal Medulla to the Right Caudate Nucleus in Two Patients with Intractable Parkinson's Disease." *New England Journal of Medicine* 316: 831-34.

Malter, H.E., and J. Cohen. 1989a. "Blastocyst Formation and Hatching In Vitro Following Zona Drilling of Mouse and Human Embryos." *Gamete Research* 24: 67-80.

—. 1989b. "Embryonic Development After Microsurgical Repair of Polyspermic Human Zygotes." *Fertility and Sterility* 52: 373-80.

—. 1989c. "Partial Zona Dissection of the Human Oocyte: A Nontraumatic Method Using Micromanipulation to Assist Zona Penetration." *Fertility and Sterility* 51: 139-48.

Malter, H., et al. 1989. "Monospermmy and Polyspermmy After Partial Zona Dissection of Reinseminated Human Oocytes." *Gamete Research* 23: 377-86.

Menkin, M.F., and J. Rock. 1948. "In Vitro Fertilization and Cleavage of Human Ovarian Eggs." *American Journal of Obstetrics and Gynecology* 55: 440-52.

Messinis, I.E., and A. Templeton. 1988. "The Effect of Antiprogestin Mifepristone (RU 486) on Maturation and In-Vitro Fertilization of Human Oocytes." *British Journal of Obstetrics and Gynaecology* 95: 592-95.

Michelmann, H.W., and L. Mettler. 1985. "Cytogenetic Investigations on Human Oocytes and Early Human Embryonic Stages." *Fertility and Sterility* 43: 320-22.

Moore, K.L. 1988. *The Developing Human: Clinically Oriented Embryology*. 4th ed. Philadelphia: W.B. Saunders.

Ng, S.C., et al. 1988. "Pregnancy After Transfer of Sperm Under Zona." *Lancet* (1 October): 790.

—. 1989. "Transfer of Human Sperm into the Perivitelline Space of Human Oocytes After Zona-Drilling or Zona-Puncture." *Fertility and Sterility* 52: 73-78.

O'Reilly, R.J., et al. 1988. "Fetal Liver Transplantation in Man." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

Penketh, R., and A. McLaren. 1987. "Prospects for Prenatal Diagnosis During Preimplantation Human Development." *Baillière's Clinical Obstetrics and Gynaecology* 1: 747-64.

Penketh, R.J.A., et al. 1989. "Rapid Sexing of Human Embryos by Non-Radioactive *In Situ* Hybridization: Potential for Preimplantation Diagnosis of X-Linked Disorders." *Prenatal Diagnosis* 9: 489-99.

Pickering, S.J., P.R. Braude, and M.H. Johnson. 1991. "Cryoprotection of Human Oocytes: Inappropriate Exposure to DMSO Reduces Fertilization Rates." *Human Reproduction* 6: 142-43.

Pickering, S.J., et al. 1990. "Transient Cooling to Room Temperature Can Cause Irreversible Disruption of the Meiotic Spindle in the Human Oocyte." *Fertility and Sterility* 54: 102-108.

—. 1992. "Reliability of Detection by Polymerase Chain Reaction of the Sickle Cell-Containing Region of the Beta-Globin Gene in Single Human Blastomeres." *Human Reproduction* 7: 630-36.

Plachot, M., et al. 1988. "Are Clinical and Biological IVF Parameters Correlated with Chromosomal Disorders in Early Life: A Multicentric Study." *Human Reproduction* 3: 627-35.

Rawlins, R.G., et al. 1988. "Microsurgical Enucleation of Tripronuclear Human Zygotes." *Fertility and Sterility* 50: 266-72.

Redmond, D.E., et al. 1988. "Cryopreservation, Culture and Transplantation of Human Fetal Mesencephalic Tissue into Monkeys." *Science* 242: 768-71.

Saiki, R.K., et al. 1988. "Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase." *Science* 239: 487-91.

Sathananthan, A.H., et al. 1989. "Human Micro-Insemination by Injection of Single or Multiple Sperm: Ultrastructure." *Human Reproduction* 4: 574-83.

Shettles, L.B. 1955. "A Morula Stage of Human Ovum Developed In Vitro." *Fertility and Sterility* 6: 287-89.

Sladek, J.R., and I. Shoulson. 1988. "Neural Transplantation: A Call for Patience Rather than Patients." *Science* 240: 1386-88.

Spain. 1988. Law 42/88, of 28th December, on the Donation and Utilization of Human Fetus and Embryos or of Their Cells, Tissues and Organs.

Strom, C.M., et al. 1990. "Preconception Genetic Diagnosis of Cystic Fibrosis." *Lancet* (4 August): 306-307.

Stromberg, I., et al. 1986. "Human Fetal Substantia Nigra Grafted to the Dopamine-Denervated Striatum of Immunosuppressed Rats: Evidence for Functional Reinnervation." *Neuroscience Letters* 71: 271-76.

Templeton, A.A., et al. 1986. "Oocyte Recovery and Fertilization Rates in Women at Various Times After the Administration of hCG." *Journal of Reproduction and Fertility* 76: 771-78.

Testart, J., et al. 1986. "High Pregnancy Rate After Early Human Embryo Freezing." *Fertility and Sterility* 46: 268-72.

Trounson, A., and L. Mohr. 1983. "Human Pregnancy Following Cryopreservation, Thawing and Transfer of an Eight Cell Embryo." *Nature* 305: 707-709.

United Kingdom. Human Fertilisation and Embryology Act. 1990, c. 37.

United Kingdom. Advisory Group on the Use of Fetuses and Fetal Material for Research. 1972. *Report*. London: Her Majesty's Stationery Office. (Sir John Peel, Chair.)

United Kingdom. Committee of Inquiry into Human Fertilisation and Embryology. 1984. *Report*. Cmnd. 9314. London: Her Majesty's Stationery Office. (Dame Mary Warnock, Chair.)

United Kingdom. Committee to Review the Guidance on the Research Use of Fetuses and Fetal Material. 1989. *Report*. Cm 762. London: Her Majesty's Stationery Office. (Rev. John Polkinghorne, Chair.)

United Kingdom. Department of Health and Social Security. 1987. *Human Fertilisation and Embryology: A Framework for Legislation*. London: Her Majesty's Stationery Office.

United States. National Institutes of Health. Human Fetal Tissue Transplantation Research Panel. 1988. *Report*. 2 vols. Bethesda: National Institutes of Health.

van Uem, J.F.H.M., et al. 1987. "Birth After Cryopreservation of Unfertilised Oocytes." *Lancet* (28 March): 752-53.

Varawalla, N.Y., et al. 1991. "An Approach to Preimplantation Diagnosis of β -Thalassaemia." *Prenatal Diagnosis* 11: 775-85.

Verlinsky, Y., et al. 1990. "Analysis of the First Polar Body: Preconception Genetic Diagnosis." *Human Reproduction* 5: 826-29.

—. 1992. "Preconception and Preimplantation Diagnosis for Cystic Fibrosis." *Prenatal Diagnosis* 12: 103-10.

Wales, R.G., et al. 1987. "Metabolism of Glucose by Human Embryos." *Journal of Reproduction and Fertility* 79: 289-97.

West, J.D. 1989. "The Use of DNA Probes in Preimplantation and Prenatal Diagnosis." *Molecular Reproduction and Development* 1: 138-45.

West, J.D., et al. 1987. "Sexing the Human Pre-Embryo by DNA-DNA In Situ Hybridisation." *Lancet* (13 June): 1345-47.

—. 1989. "Glucose Phosphate Isomerase Activity in Mouse and Human Eggs and Pre-Embryos." *Human Reproduction* 4: 82-85.

Wilson, K.M. 1945. "A Normal Human Ovum of Sixteen Days Development — The Rochester Ovum." *Contributions to Embryology* 31: 101-106.

Wimmers, M.S.E., and J.V. Van der Merwe. 1988. "Chromosome Studies on Early Human Embryos Fertilized *In Vitro*." *Human Reproduction* 3: 894-900.

Winston, N.J., et al. 1991. "The Incidence of Abnormal Morphology and Nucleocytoplasmic Ratios in 2-, 3- and 5-Day Human Pre-Embryos." *Human Reproduction* 6: 17-24.

Wong, L.C. 1988. "Medical Research Council Tissue Bank." In *Report of the Human Fetal Tissue Transplantation Research Panel*, vol. 2. Bethesda: National Institutes of Health.

Yamamoto, M., and G. Watanabe. 1979. "Epidemiology of Gross Chromosomal Anomalies at the Early Embryonic Stage of Pregnancy." In *Epidemiologic Methods for Detection of Teratogens*, ed. M.A. Klinberg and J.A.C. Weatherall. Basel: Karger.

Zeilmaker, G.H., et al. 1984. "Two Pregnancies Following Transfer of Intact Frozen-Thawed Embryos." *Fertility and Sterility* 42: 293-96.

Contributors

K.J. Betteridge, M.V.Sc., Ph.D., FRCVS, Animal Biotechnology Embryo Laboratory, Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph.

Bernard M. Dickens, Ph.D., LL.D., Faculty of Law and Faculty of Medicine, University of Toronto.

Alan Fine, V.M.D., Ph.D., Faculty of Medicine, Dalhousie University.

Anne McLaren, D.Phil., FRS, FRCOG, DBE.

Michelle A. Mullen, B.Sc., M.H.P., Centre for Bioethics, University of Toronto.

D. Rieger, B.Sc., Ph.D., Animal Biotechnology Embryo Laboratory, Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph.

SPR Associates Inc., Toronto, Ontario.

Mandate

(approved by Her Excellency the Governor General
on the 25th day of October, 1989)

The Committee of the Privy Council, on the recommendation of the Prime Minister, advise that a Commission do issue under Part I of the Inquiries Act and under the Great Seal of Canada appointing The Royal Commission on New Reproductive Technologies to inquire into and report on current and potential medical and scientific developments related to new reproductive technologies, considering in particular their social, ethical, health, research, legal and economic implications and the public interest, recommending what policies and safeguards should be applied, and examining in particular,

- (a) implications of new reproductive technologies for women's reproductive health and well-being;
- (b) the causes, treatment and prevention of male and female infertility;
- (c) reversals of sterilization procedures, artificial insemination, *in vitro* fertilization, embryo transfers, prenatal screening and diagnostic techniques, genetic manipulation and therapeutic interventions to correct genetic anomalies, sex selection techniques, embryo experimentation and fetal tissue transplants;
- (d) social and legal arrangements, such as surrogate childbearing, judicial interventions during gestation and birth, and "ownership" of ova, sperm, embryos and fetal tissue;
- (e) the status and rights of people using or contributing to reproductive services, such as access to procedures, "rights" to parenthood, informed consent, status of gamete donors and confidentiality, and the impact of these services on all concerned parties, particularly the children; and
- (f) the economic ramifications of these technologies, such as the commercial marketing of ova, sperm and embryos, the application of patent law, and the funding of research and procedures including infertility treatment.

The Research Volumes

Volume 1: New Reproductive Technologies: Ethical Aspects

Approaches to the Ethical Issues Raised by the
Royal Commission's Mandate

W. Kymlicka

Assisted Reproductive Technologies:
Informed Choice

F. Baylis

Medicalization and the New Reproductive
Technologies

M. Burgess/A. Frank/
S. Sherwin

Prenatal Diagnosis and Society

D.C. Wertz

Roles for Ethics Committees in Relation to
Guidelines for New Reproductive
Technologies: A Research Position Paper

J.B. Dossetor/J.L. Storch

Economic, Ethical, and Population Aspects of
New Reproductive Technologies in
Developing Countries: Implications for
Canada

P. Manga

Volume 2: Social Values and Attitudes Surrounding New Reproductive Technologies

An Overview of Findings in This Volume

RCNRT Staff

Social Values and Attitudes of Canadians
Toward New Reproductive Technologies

Decima Research

Social Values and Attitudes of Canadians
Toward New Reproductive Technologies:
Focus Group Findings

Decima Research

Key Findings from a National Survey Conducted
by the Angus Reid Group: Infertility,
Surrogacy, Fetal Tissue Research, and
Reproductive Technologies

M. de Groh

Reproductive Technologies, Adoption, and Issues on the Cost of Health Care: Summary of Canada Health Monitor Results	M. de Groh
Survey of Ethnocultural Communities on New Reproductive Technologies	S. Dutt
World Religions and New Reproductive Technologies	H. Coward
Personal Experiences with New Reproductive Technologies: Report from Private Sessions	RCNRT Staff

Volume 3: Overview of Legal Issues in New Reproductive Technologies

The Constitution and the Regulation of New Reproductive Technologies	M. Jackman
An Overview of the Legal System in Canada	S.L. Martin
Overview of Canadian Laws Relating to Privacy and Confidentiality in the Medical Context	E.L. Oscapella
Reproductive Technology: Is a Property Law Regime Appropriate?	M.M. Litman/ G.B. Robertson
New Reproductive Technologies: Commercial Protection	K.M. Cherniawsky/ P.J.M. Lown
The Limits of Freedom of Contract: The Commercialization of Reproductive Materials and Services	M. Martin/A. Lawson/ P. Lewis/M. Trebilcock
Appropriating the Human Being: An Essay on the Appropriation of the Human Body and of Its Parts	J. Goulet
The Civil Code of Québec and New Reproductive Technologies	M. Ouellette
New Reproductive Technologies: International Legal Issues and Instruments	R.J. Cook

Volume 4: Legal and Ethical Issues in New Reproductive Technologies: Pregnancy and Parenthood

Juridical Interference with Gestation and Birth	S. Rodgers
Reproductive Hazards in the Workplace: Legal Issues of Regulation, Enforcement, and Redress	J. Fudge/E. Tucker
The Challenge of the New Reproductive Technologies to Family Law	E. Sloss/R. Mykitiuk
"Surrogate Motherhood": Legal and Ethical Analysis	J.R. Guichon
Surrogate Parenting: Bibliography	J. Kitts

Volume 5: New Reproductive Technologies and the Science, Industry, Education, and Social Welfare Systems in Canada

Discovery, Community, and Profit: An Overview of the Science and Technology System	L. Edwards, with the assistance of R. Voyer
An Overview of Select Social and Economic Forces Influencing the Development of <i>In Vitro</i> Fertilization and Related Assisted Reproductive Techniques	A. Rochon Ford
Commercial Involvement in New Reproductive Technologies: An Overview	J. Rowlands/N. Saby/J. Smith
The Role of the Biotechnology Industry in the Development of Clinical Diagnostic Materials for Prenatal Diagnosis	G. Chaloner-Larsson/F. Haynes/C. Merritt
Report on a Survey of Members of the Pharmaceutical Manufacturers Association of Canada and Biotechnology Companies	SPR Associates Inc.
Canada's School Systems: An Overview of Their Potential Role in Promoting Reproductive Health and Understanding of New Reproductive Technologies	Shannon and McCall Consulting Ltd.
Social Welfare and New Reproductive Technologies: An Overview	S. Torjman

Volume 6: The Prevalence of Infertility in Canada

Historical Overview of Medical Perceptions of Infertility in Canada, 1850-1950

W.L. Mitchinson

The Prevalence of Infertility in Canada, 1991-1992: Analysis of Three National Surveys

C.S. Dulberg/T. Stephens

Infertility Among Canadians: An Analysis of Data from the Canadian Fertility Survey (1984) and General Social Survey (1990)

T.R. Balakrishnan/
R. Fernando

Infertility, Sterilization, and Contraceptive Use in Ontario

T.R. Balakrishnan/
P. Maxim

Adoption as an Alternative for Infertile Couples: Prospects and Trends

K.J. Daly/M.P. Sobol

Annotated Bibliography on the Prevalence of Infertility

M.R.P. de la Roche

Volume 7: Understanding Infertility: Risk Factors Affecting Fertility

Sexually Transmitted Infections: Their Manifestations and Links to Infertility and Reproductive Illness

A.R. Ronald/R.W. Peeling

The Physiological Effects of Aging on Fertility Decline: A Literature Review

J. Jantz-Lee

Effects of Licit and Illicit Drugs, Alcohol, Caffeine, and Nicotine on Infertility

H. Boyer

A Literature Review of the Physiological Manifestations Related to Infertility Linked to Weight, Eating Behaviours, and Exercise

S.E. Maddocks

Contraception: An Evaluation of Its Role in Relation to Infertility — Can It Protect?

B.N. Barwin/W. Fisher

The Physiological Links Between Endometriosis and Infertility: Review of the Medical Literature and Annotated Bibliography (1985-1990)

A. Ponchuk

The Impact of Medical Procedures on Fertility

S. Dumas/

É. Guilbert/J-É. Rioux

**Occupational and Environmental Exposure Data:
Information Sources and Linkage Potential
to Adverse Reproductive Outcomes Data in
Canada**

P.K. Abeytunga/
M. Tennessee

**Evaluation of an Environmental Contaminant:
Development of a Method for Chemical
Review and a Case Study of
Hexachlorobenzene (HCB) as a
Reproductive Toxicant**

J.F. Jarrell/
J. Seidel/P. Bigelow

**Pilot Study on Determining the Relative
Importance of Risk Factors for Infertility in
Canada**

P. Millson/K. Maznyk

Volume 8: Prevention of Infertility

**Prevention of Infertility: Overcoming the
Obstacles**

A. Thomson

**The Effectiveness of Sexually Transmitted
Disease Infertility-Related Prevention
Programs**

L. McIntyre

**The Burden of Chlamydial and Gonococcal
Infection in Canada**

R. Goeree/P. Gully

**Social Factors Relevant to Sexually Transmitted
Diseases and to Strategies for Their
Prevention: A Literature Review**

L. Hanvey/D. Kinnon

**Feasibility of Economic Evaluations of Sexually
Transmitted Disease Prevention Programs in
Canada**

R. Goeree

**Issues in Evaluating Programs to Prevent
Infertility Related to Occupational Hazards**

A. Yassi

**The Integration of Theoretical Approaches to
Prevention: A Proposed Framework for
Reducing the Incidence of Infertility**

B. Hyndman/A. Libstug/
I. Rootman/N. Giesbrecht/
R. Osborn

Volume 9: Treatment of Infertility: Assisted Reproductive Technologies

Part 1: Overview of Assisted Reproductive Technologies

Medically Assisted Reproductive Technologies:
A Review

M.A. Mullen

A Socio-Historical Examination of the
Development of *In Vitro* Fertilization and
Related Assisted Reproductive Techniques

A. Rochon Ford

The Professions Involved in New Reproductive
Technologies: Their Present and Future
Numbers, Training, and Improvement in
Competence

L. Curry

Legislation, Inquiries, and Guidelines on
Infertility Treatment and
Surrogacy/Preconception Contracts: A
Review of Policies in Seven Countries

L.S. Williams

Part 2: Assisted Insemination

Donor Insemination: An Overview

R. Achilles

Issues and Responses: Artificial Insemination

D. Wikler/N. Wikler

The Social Meanings of Donor Insemination

R. Achilles

Lesbian Women and Donor Insemination:
An Alberta Case Study

F.A.L. Nelson

Self-Insemination in Canada

R. Achilles

The Conceptual Framework of Donor
Insemination

D. Wikler

Artificial Insemination: Bibliography

M. Musgrove

Volume 10: Treatment of Infertility: Current Practices and Psychosocial Implications

Survey of Canadian Fertility Programs

T. Stephens/J. McLean,
with R. Achilles/L. Brunet/
J. Wood Catano

An Evaluation of Canadian Fertility Clinics:
The Patient's Perspective

SPR Associates Inc.

Infertile Couples and Their Treatment in Canadian Academic Infertility Clinics

J. Collins/E. Burrows/
A. Willan

Implementing Shared Patient Decision Making: A Review of the Literature

R.B. Deber, with
H. Bouchard/A. Pendleton

The Psychosocial Impact of New Reproductive Technology

J. Wright

Life Quality, Psychosocial Factors, and Infertility: Selected Results from a Five-Year Study of 275 Couples

A. Abbey/L.J. Halman/
F.M. Andrews

Review of the Literature on the Psychosocial Implications of Infertility Treatment on Women and Men

E. Savard Muir

Volume 11: New Reproductive Technologies and the Health Care System: The Case for Evidence-Based Medicine

The Canadian Health Care System

M.M. Rachlis

Framework for Technology Decisions:
Literature Review

A. Kazanjian/K. Cardiff

Infertility Treatment: From Cookery to Science — The Epidemiology of Randomized Controlled Trials

P. Vandekerckhove/
P.A. O'Donovan/
R.J. Lilford/T.W. Harada

Meta-Analysis of Controlled Trials in Infertility

E.G. Hughes/

Treatment of Male Infertility: Is It Effective? A Review and Meta-Analyses of Published Randomized Controlled Trials

D.M. Fedorkow/J.A. Collins

Adverse Health Effects of Drugs Used for Ovulation Induction

P. Vandekerckhove/
P.A. O'Donovan/
R.J. Lilford/E. Hughes

Methodological Challenges in Evaluating a New and Evolving Technology: The Case of *In Vitro* Fertilization

J.F. Jarrell/J. Seidel/
P. Bigelow

Cost-Effectiveness of an *In Vitro* Fertilization Program and the Costs of Associated Hospitalizations and Other Infertility Treatments

R. Goeree/J. Jarrell/
R. Labelle

R. Goeree/R. Labelle/
J. Jarrell/R. Milner

Public Preferences Toward an *In Vitro* Fertilization Program and the Effect of the Program on Patients' Quality of Life

The Child Health Study: Record Linkage Feasibility of Selected Data Bases: A Catalogue

Infertility Treatment — Epidemiology, Efficacy, Outcomes, and Direct Costs: A Feasibility Study, Saskatchewan 1978-1990

R. Goeree/R. Labelle/
J. Jarrell

L. Hayward/D.E. Flett/
C. Davis

C. D'Arcy/N.S.B. Rawson/
L. Edouard

Volume 12: Prenatal Diagnosis: Background and Impact on Individuals

The History and Evolution of Prenatal Diagnosis

Risk Assessment of Prenatal Diagnostic Techniques

A Survey of Research on Post-Natal Medical and Psychological Effects of Prenatal Diagnosis on Offspring

A Demographic and Geographic Analysis of the Users of Prenatal Diagnostic Services in Canada

Perceptions, Attitudes, and Experiences of Prenatal Diagnosis: A Winnipeg Study of Women Over 35

Manitoba Voices: A Qualitative Study of Women's Experiences with Technology in Pregnancy

A Review of Views Critical of Prenatal Diagnosis and Its Impact on Attitudes Toward Persons with Disabilities

Parental Reaction and Adaptability to the Prenatal Diagnosis of Genetic Disease Leading to Pregnancy Termination

I.F. MacKay/F.C. Fraser

RCNRT Staff

J. Beck

P.M. MacLeod/
M.W. Rosenberg/
M.H. Butler/S.J. Koval

K.R. Grant

S. Tudiver

J. Milner

L. Dallaire/G. Lortie

Volume 13: Current Practice of Prenatal Diagnosis in Canada

Prenatal Diagnosis in Canada — 1990:
A Review of Genetics Centres

J.L. Hamerton/
J.A. Evans/L. Stranc

An Assessment of the Readability of Patient Education Materials Used by Genetic Screening Clinics

J. Wood Catano

Canadian Physicians and Prenatal Diagnosis: Prudence and Ambivalence

M. Renaud/L. Bouchard/
J. Bisson/J-F. Labadie/
L. Dallaire/N. Kishchuk

An Analysis of Temporal and Regional Trends in the Use of Prenatal Ultrasonography

G.M. Anderson

Maternal Serum AFP Screening Programs:
The Manitoba Experience

B.N. Chodirker/J.A. Evans

Volume 14: Technologies of Sex Selection and Prenatal Diagnosis

Ethical Issues of Prenatal Diagnosis for Predictive Testing for Genetic Disorders of Late Onset

M. Cooke

Prenatal Testing for Huntington Disease:
Psychosocial Aspects

S. Adam/M.R. Hayden

Screening for Genetic Susceptibilities to Common Diseases

L. Prior

Preference for the Sex of One's Children and the Prospective Use of Sex Selection

M. Thomas

Bibliography on Preferences for the Sex of One's Children, and Attitudes Concerning Sex Preselection

M. Thomas

Attitudes of Genetic Counsellors with Respect to Prenatal Diagnosis of Sex for Non-Medical Reasons

Z.G. Miller/F.C. Fraser

Preimplantation Diagnosis

F.C. Fraser

Somatic and Germ Line Gene Therapy:
Current Status and Prospects

L. Prior

Volume 15: Background and Current Practice of Fetal Tissue and Embryo Research in Canada

The Use of Human Embryos and Fetal Tissues: A Research Architecture	M.A. Mullen
Legal Issues in Embryo and Fetal Tissue Research and Therapy	B.M. Dickens
Human Fetal Tissue Research: Origins, State of the Art, Future Applications, and Implications	A. Fine
Report on a Survey of Use and Handling of Human Reproductive Tissues in Canadian Health Care Facilities	SPR Associates Inc.
Report on a Follow-Up Survey of Use and Handling of Human Reproductive Tissues (Survey of Medical Laboratories and Medical Waste Disposal Firms)	SPR Associates Inc.
Embryo Transfer and Related Technologies in Domestic Animals: Their History, Current Status, and Future Direction, with Special Reference to Implications for Human Medicine	K.J. Betteridge/D. Rieger
Human Embryo Research: Past, Present, and Future	A. McLaren

Commission Organization

Commissioners

Patricia Baird

Chairperson

Vancouver, British Columbia

Grace Jantzen

London, United Kingdom

Bartha Maria Knoppers

Montreal, Quebec

Susan E.M. McCutcheon

Toronto, Ontario

Suzanne Rozell Scorsone

Toronto, Ontario

Staff

John Sinclair

Executive Director

Mimsie Rodrigue

Executive Director (from July 1993)

Research & Evaluation

Sylvia Gold

Director

Nancy Miller Chénier

Deputy Director

Causes and Prevention of Infertility

Janet Hatcher Roberts

Deputy Director

Assisted Human Reproduction

F. Clarke Fraser

Deputy Director

Prenatal Diagnosis and Genetics

Burleigh Trevor Deutsch

Deputy Director

Embryo and Fetal Tissue Research

Consultations & Coordination

Dann M. Michols

Director

Mimsie Rodrigue

Deputy Director

Coordination

Anne Marie Smart

Deputy Director

Communications

Judith Nolté

Deputy Director

Analysis

Denise Cole

Deputy Director

Consultations

Mary Ann Allen

Director

Administration and Security

Gary Paradis

Deputy Director

Finance

3416

ISBN 0-662-21389-0

A standard linear barcode representing the ISBN number 0-662-21389-0.

9 780662 213895

